Cardiac Syndrome X, Insulin Resistance and Microvascular Dysfunction – the use of Metformin in a Double-Blind Randomised Controlled Trial

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Declarations:

This M.D. thesis has been composed entirely by Dr Sachin Jadhav

All of the work carried out in this research project has been done solely by Dr Sachin Jadhav within the departments of Medical Cardiology and Pathological Biochemistry, Glasgow Royal Infirmary, Glasgow from February 2000 to August 2002.

Dr Sachin Jadhav holds the degree of MBChB (Hons) from the University of Edinburgh 1996.

This thesis and work therein has not been submitted for any other degree, postgraduate diploma or professional qualification.

This thesis contains published manuscripts within appendix 3 and abstracts within appendix 4 – permission of co-authors has been obtained for inclusion within this thesis.

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ABSTRACT OF THESIS

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The syndrome of cardiac chest pain with a positive exercise tolerance test but angiographically normal coronary arteries (cardiac Syndrome X) is a common clinical scenario. There is now a good evidence base, suggesting that these patients are relatively insulin resistant and have abnormalities of coronary and peripheral vasomotor function. I begin by examining the background of insulin resistance along with its link to endothelial function, its measurement and potential aetiology along with its established link to atheromatous coronary disease. Chapter 2 introduces cardiac 'Syndrome X' by looking, in a balanced way, at the evidence for ischaemia, as well as the evidence for insulin resistance and endothelial function in these patients. I postulate that underlying insulin resistance in these patients leads to general abnormalities of vascular function and therefore reduced vasodilator capacity in the coronary bed as the substrate for myocardial ischaemia.

I performed a study in which women with cardiac 'Syndrome X' were initially compared with a healthy control group to examine differences in their metabolic measures, anthropometric measures and peripheral microvascular function. I then went on to perform a randomised double-blinded placebo-controlled trial during which metformin or placebo was administered for 8 weeks to women with cardiac 'Syndrome X'. The recruitment protocol and methods used are described in chapter 3.

The differences between women with cardiac 'Syndrome X' and healthy controls are discussed in chapter 4. I show that after correction for age and body mass index, there exists significant differences between the groups in terms of indices of insulin resistance, some lipid parameters, some serum markers of endothelial function and serum leptin.

The process of laser Doppler imaging in conjunction with iontophoretically-applied acetyl-choline and sodium nitroprusside is used to assess peripheral microvascular function, and this is discussed in chapter 5. In particular, the reproducibility of these measurements both between arms, and several weeks apart is validated. I go on to show that there is a significant difference both in the endothelium-dependent and independent peripheral microvascular vasodilating response, between women with cardiac 'Syndrome X' and controls, in chapter 6.

I demonstrate in chapter 7 that administration of metformin to women with cardiac 'Syndrome X' results in significant improvement in some indices of insulin resistance, some lipid measures, some serum markers of endothelial function and body mass. Furthermore, in chapter 8, improvement in endothelium-dependent microvascular function and in some ischaemic-measures is shown following metformin administration. These findings are summarised and discussed in chapter 9.

Outline of Thesis

FOREWORD

There is mounting evidence that hyperinsulinaemia, as a surrogate marker for insulin resistance, is an independent cardiovascular risk factor. As well as the promotion of atherogenesis, there is evidence to suggest that insulin resistance has a role in the pathogenesis of vasomotor disturbances often seen in tandem with atheromatous plaques. It is known that insulin is important in the endothelial production of nitric oxide and abnormal vasomotor function may be related to blunted nitric oxide production, at least in part, which would manifest in an insulin resistant state.

Cardiac 'Syndrome X' is a label used to describe patients with ischaemic-type chest pain, indirect evidence of myocardial ischaemia and unobstructed coronary arteries. These patients form a heterogeneous group and although a significant proportion has non-cardiac chest pain, there is good evidence to suggest that at least a subgroup has genuine myocardial ischaemia. Theories regarding the aetiology of ischaemia in these patients include reduced vasodilator reserve as a result of microvascular endothelial dysfunction. This may be related to insulin resistance, which has been demonstrated in patients with 'Syndrome X'.

Chapter one of this thesis introduces the concept of insulin resistance and its measurement. The current level of knowledge regarding intracellular insulin signalling and the potential mechanisms for the aetiology of insulin resistance are presented. Finally, the relationship between insulin resistance, general atheroma and coronary artery disease is examined with the final section focusing on the prospective data looking at the risk of coronary heart disease with surrogate markers of insulin resistance.

Chapter two discusses cardiac 'Syndrome X' and microvascular angina looking at the evidence for an ischaemic basis and potential causes of non-cardiac pain. Various clinical tools for non-invasive demonstration of ischaemia are discussed. The potential aetiology and pathogenesis are reviewed with reference to insulin resistance, oestrogen deficiency and vascular dysfunction. This culminates with the hypothesis that patients with microvascular angina are insulin resistant leading to abnormal vascular function which is responsible for the generation of ischaemia. Following on from this, insulin sensitisation should improve markers of insulin resistance, vascular function and perhaps even symptoms in this patient group.

Chapter three then follows on to describe the recruitment, study protocol and methods used within the double-blind placebo-controlled trial looking at :

- the differences between women with microvascular angina and normal control subjects, in terms of metabolic parameters, risk factor profile and peripheral microvascular function.

- effects of 8 weeks of metformin therapy in women with microvascular angina in terms of their microvascular function, features of the metabolic syndrome and ischaemic measures/symptoms.

Chapter four presents some of the differences between the cohort of women with 'Syndrome X' at baseline and a group of healthy controls. Data on clinical, anthropometric and metabolic variables are presented in this chapter.

Chapter five introduces the concept of measurement of peripheral vascular function and specifically expands upon the methodology of laser Doppler imaging in conjunction with iontophoresis of vasoactive substances, which was the method used in the trial. Data showing the reproducibility of this method are presented. Chapter six deals with the differences seen in peripheral microvascular function between healthy controls and the group of women with 'Syndrome X' and looks at some of the correlations which exist between peripheral vascular function and some metabolic variables.

The impact of metformin upon clinical, metabolic and anthropometric measures is presented in chapter seven and chapter eight deals with the differences seen in peripheral microvascular function following treatment with metformin.

Finally chapter nine summarises the results and discusses the implications this double blind randomised controlled trial.

CHAPTER 1

Insulin Resistance – Measurement, Aetiology and Link to Cardiovascular Disease

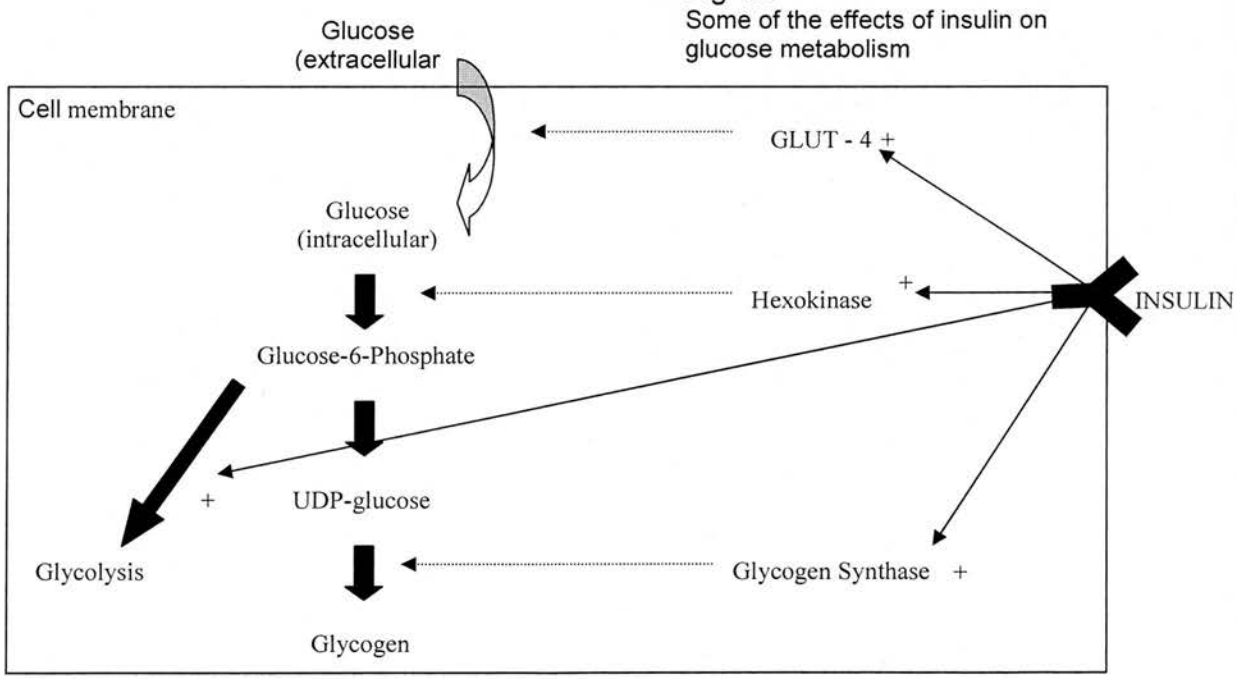
Introduction

Insulin Resistance

Insulin resistance is pivotal in the pathogenesis of type 2 diabetes mellitus. It has also been described in several other conditions, to varying extents, including polycystic ovarian syndrome, Cushings syndrome, exogenous steroid therapy, obesity and pregnancy. The importance of type 2 diabetes mellitus is well established as a cardiovascular risk factor, but only over the last 2 decades has the risk of patients with subclinical glucose intolerance and even normal glucose tolerance but insulin resistance, been investigated.

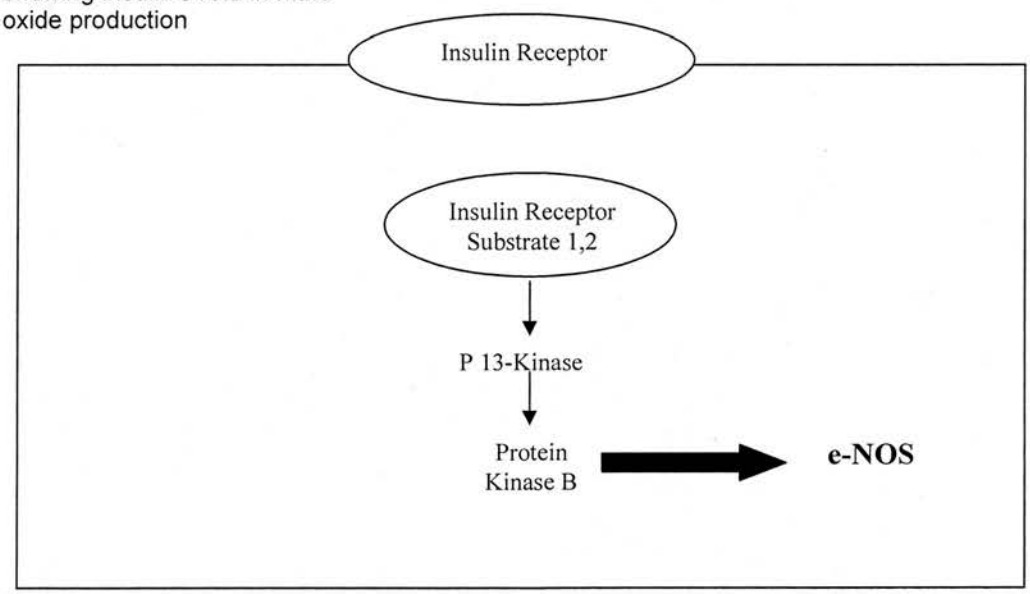
The term 'insulin insensitivity' was first used by Himsworth and Kerr in 1939 when distinguishing between type 1 and type 2 diabetes mellitus (1). The term 'insulin resistance' refers to an impaired biological response to insulin. The most well known in-vivo effect of insulin is its effect on glucose metabolism, some of which is illustrated below in figure 1.1:

Fig 1.1
Some of the effects of insulin on glucose metabolism



Despite several effects on glucose metabolism, insulin resistance is a term that usually refers specifically to reductions in insulin-mediated glucose uptake. However, this is only one function of this peptide hormone, and its actions on glucose and lipid metabolism reach much further. One of the most important roles of insulin in terms of its relation to cardiovascular disease is thought to be its influence on the endothelial cell monolayer. The endothelium is intimately involved in vascular homeostasis through its many functions. One of the most important is the modulation of vascular tone via mediators including nitric oxide (NO). Insulin appears to have a critical role in this modulation with a significant local and systemic vasodilating effect. Insulin-mediated vasodilation has been shown to correlate with whole body insulin sensitivity in healthy subjects (2). Furthermore, this property does not seem to be independent of insulin's effect on glucose metabolism. The vasodilating effect of insulin is significantly augmented when glucose is co-infused, as measured by plethysmography and this suggests that the vascular and metabolic effects of insulin are closely coupled (3). A simplified pathway for its effect on NO is shown in figure 1.2 below.

Fig 1.2
Part of intra-cellular pathway showing insulin's role in nitric oxide production



P 13-Kinase – Phosphatidyl inositol 3-kinase
e-NOS – endothelial nitric oxide synthase

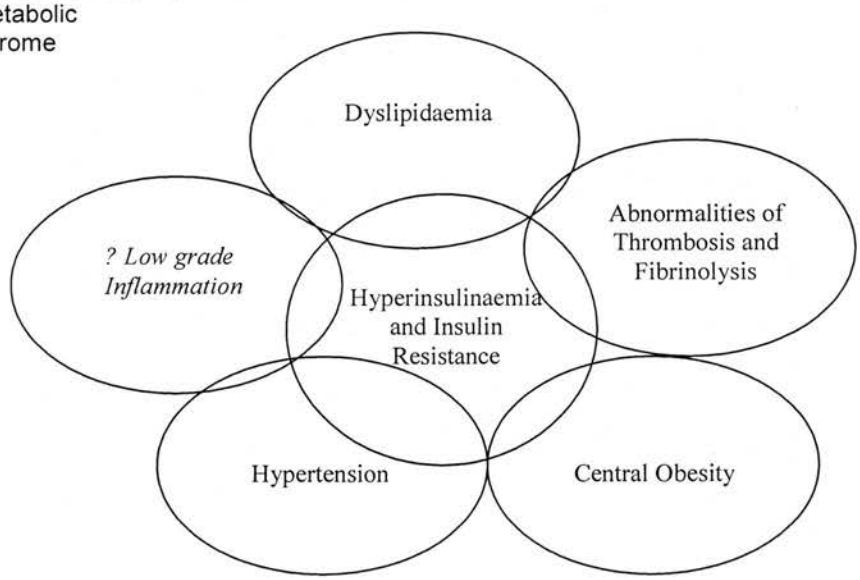
The vasodilating effect of insulin is manifest via intermediates including nitric oxide (NO) on the cellular level. Indeed, correlations in healthy individuals, between whole body insulin sensitivity (in terms of insulin-mediated glucose uptake) and basal NO production, as demonstrated by vasoconstrictor responses to N-monomethyl-L-arginine (L-NMMA), have been described (4). This vascular relationship of insulin to endothelial dysfunction mediated by impaired NO production may have an important position in the pathogenesis of cardiovascular disease, the incidence of which is increased in insulin resistant states (5).

The Metabolic syndrome

The metabolic syndrome, now also called the 'Insulin resistance Syndrome' was first described by Reaven almost 30 years ago, but since that time the definition has been refined and augmented (6). It is now clear that it consists of a cluster of inter-related cardiovascular risk factors including hypertension, dyslipidaemia, central obesity and disturbances of fibrinolysis with abnormalities of glucose and insulin metabolism at the core, as illustrated in figure 1.3. Low-grade systemic inflammation has more recently been linked with cardiovascular events using the marker of serum C-reactive protein (CRP) (7). CRP also has associations with other features of the insulin resistance syndrome (8) and some have argued for its inclusion as part of the syndrome (9).

Data exist showing that subjects with the metabolic syndrome have a higher risk of cardiovascular disease and an increased cardiovascular mortality (10). This would be expected given the various cardiovascular risk factors encompassed. However, hyperinsulinaemia, a marker of insulin resistance, is also shown to confer independent increased cardiovascular risk prospectively (11). This is examined in more detail.

Fig 1.3
Schematic of features of Metabolic Syndrome



Measuring Insulin Resistance

The gold standard technique for measuring insulin resistance is the hyperinsulinaemic euglycaemic clamp technique, developed by Andres et al (12). Exogenous insulin is infused intravenously at a fixed rate along with glucose and the rate of glucose infusion is adjusted frequently to keep the serum glucose at a steady state. The amount of glucose infused over a unit time reflects whole body insulin-mediated glucose uptake and as such is a measure of insulin sensitivity (M). This technique is a research tool and is cumbersome, time consuming, expensive and fairly invasive to use. Furthermore, it is a non-physiological model.

A less invasive method of quantifying insulin resistance is that termed the minimal model. This involves an intravenous glucose tolerance test with frequent measures of serum glucose and insulin, usually via an indwelling venous catheter. A calculated index of insulin sensitivity (S_i) is generated on entering these values into a computer model (13). There is reasonably good correlation between S_i and M provided subjects do not have diabetes (for reasons stated below). Although this method is more practical, it remains time-consuming and relatively expensive and therefore, it too has a very restricted role outside of research.

A method termed the 'Homeostasis Model Assessment' (HOMA) relies on the fact that basal serum insulin and glucose concentrations are determined by a feedback loop involving systemic insulin resistance and islet beta-cell function. Fasting insulin and glucose are measured and input into the formula:

HOMA-IR = [insulin x glucose] / 22.5

This method has also been shown to have favourable correlations with clamp-derived estimates of whole body insulin sensitivity - the correlation co-efficient of variability between HOMA-IR and hyperglycaemic clamp was robust (r^2=0.69, $p<0.01$) (14).

A more clinically relevant method for calculating insulin resistance is the use of fasting or post-glucose load insulin levels. In general, as peripheral tissues become insulin resistant, beta-islet cells in the pancreas compensate by increasing insulin secretion. Therefore, higher insulin levels should be markers for systemic insulin resistance. However, this relationship breaks down after many years, and in type 2 diabetes, beta-cells that have been hyper-secreting insulin "burn out" with falls in the levels of insulin secretion. For this reason, patients with diabetes may have lower insulin levels than subjects with subclinical insulin resistance and normal glucose tolerance, despite having higher levels of systemic insulin resistance.

Despite this potentially paradoxical relationship, insulin levels do have a place in the calculation of total body insulin resistance. It has been shown that when subjects are divided into categories of glucose tolerance status, namely normal, impaired or type 2 diabetes, fasting insulin levels correlate reasonably well with formally measured insulin resistance as assessed by the clamp technique within each group (15). The correlation, as expected, is strongest in those with normal glucose tolerance, as these subjects will tend to have the most active beta-islet cells. In subjects with normal or even impaired glucose tolerance, but not in the diabetic population, post-glucose load insulin levels also correlated reasonably well with total body insulin resistance (15).

Some groups have made use of a simplified index of insulin resistance termed 'Admission Index of Insulin Resistance' (AIRI) for in-patients, based on the product of serum insulin x glucose concentrations. This has been used to calculate insulin resistance in the non-diabetic population at the time of acute hospital admission, and was adopted by Stubbs et al, in a study looking at acute coronary syndromes (16). The confounding influence of stress hormones with their physiological insulin antagonism, in this index, is potentially problematic. However, the AIRI was related to insulin levels, which were subsequently measured during an oral glucose tolerance test (17), although no direct clamp correlations are described.

It is known, however, that the relationship between fasting insulin and insulin resistance is not linear, but hyperbolic. For this reason transforming fasting insulin levels both logarithmically and reciprocally has been proposed as a more accurate surrogate measure of insulin sensitivity. An index known as the QUICKI (quantitative insulin-sensitivity check index) incorporates both of these transformations and is calculated as :

$$QUICKI = 1 / [\log(insulin) + \log(glucose)].$$

Another measure using the log (HOMA-IR) uses this mathematical principle and has been found to be useful. These are discussed further below.

The best data looking at surrogate measures of insulin resistance and their correlation to the gold-standard clamp technique is by Mather et al (18). This group looked at a total of 152 lean, obese and diabetic subjects and performed over 250 hyper-insulinaemic clamps. These data looking at the various indices described above have shown that the logarithm-transformed measures such as the QUICKI and log(HOMA-IR) produce a normal distribution unlike the measures such as HOMA, fasting insulin and reciprocal insulin measures These log-transformed measures were also accurate in terms of their repeatability, comparing favourably with repeatability of the index derived from the hyperinsulinaemic clamp.

As regards the correlation with clamp data, the fasting insulin, reciprocal insulin index (40/insulin) and the HOMA-IR did not have as good a relationship as did the log-transformed indices (log [fasting insulin] ,QUICKI and log [HOMA-IR]). Good, statistically significant, correlations between clamp indices and the QUICKI and log [HOMA-IR] in obese subjects and subjects with type 2 diabetes, were observed. These are presented in table 1.1 below. The strength of the correlation depended on the dose of insulin used in the clamp for each group. The optimal correlation is shown only, with the dose of insulin used.

Table 1.1:
Correlations between clamp-derived indices of insulin resistance and other surrogate measures (18).

	Dose of Insulin ($mU/m^2.min$)	log [insulin]	log [HOMA-IR]	QUICKI
lean subjects (n=69)	40	r=-0.41	r=-0.38	r=-0.36
obese subjects (n=53)	120	r=-0.73	r=-0.72	r=-0.73
type 2 diabetes (n=6)	600	r=-0.87	r=-0.93	r=-0.94

However, as can be seen, less good correlations (although still statistically significant) were seen in lean insulin sensitive subjects. This is thought to be a statistical consequence brought about by the greater variability of current insulin assays at the lower end of the insulin range (18). Finally, the changes in insulin resistance brought about by intervention (such as treatment with troglitazone) were seen to be as readily picked-up by the QUICKI and log [HOMA-IR] indices as the clamp. This would imply that these measures would have some use in prospective trials involving manipulation of insulin resistance.

The use of a fasting insulin level or other similar index seems to be a reasonably good surrogate marker for insulin resistance, as long as subjects are non-diabetic and comparisons are limited to subjects within the same category of glucose tolerance. However, logarithmically transformed indices such as the QUICKI and HOMA-IR appear to be superior in their normal distribution, repeatability and correlation with the hyperinsulinaemic euglycaemic clamp. It should be noted that this correlation between clamp-derived indices of insulin resistance and other surrogate measures, does seem to somewhat break down when describing insulin sensitive subjects.

The strengths and weaknesses of these various surrogate markers as presented in table 1.2.

Table 1.2: Markers of Insulin Resistance

Method	Advantages	Disadvantages
Hyperinsulinaemic euglycaemic clamp	Gold Standard	Invasive, expensive Time-consuming non-physiological
Minimal Model	Less invasive than clamp	Time-consuming Expensive
HOMA-IR	Requires only x1 fasting sample	Non-linear distribution Repeatability not as good as log-based indices
Log Transformations, QUICKI and log (HOMA-IR)	Requires only x1 fasting sample Normal distribution Good repeatability	Poor correlation with clamp in insulin sensitive population
Fasting Insulin	Requires only x1 fasting sample	Non-linear distribution Repeatability not as good as log-based indices
Reciprocal	Requires only x1 fasting sample	Non-linear distribution
AIRI	Simple – can be measured immediately with one sample	Non-linear distribution Repeatability not as good as log-based indices Confounding effects of stress hormones

The Aetiology of Insulin Resistance

The increased risk of type 2 diabetes in those with a diabetic first-degree relative is well recognised and suggests a genetic predisposition to insulin resistance. However, environmental factors play a part. For example, the observation that insulin resistance is much more prevalent in the over-weight population suggests that obesity also has a central role.

Any genetic basis for the development of insulin resistance is likely to be mediated by mutations leading to impaired insulin signalling at the molecular level. The network chain of polypeptides involved in insulin signalling at the receptor, post-receptor and gene transcription level are only poorly understood at present. However, some important aspects of the molecular biology have been identified in recent years.

Insulin signalling

The insulin receptor (InR) consists of 4 subunits : 2 extra-cellular alpha subunits to which the insulin molecule binds and 2 intracellular beta subunits. Insulin binding is known to cause phosphorylation of the beta subunits on tyrosine residues by its adjacent beta subunit. In this activated form the InR phosphorylates several other downstream proteins including the Insulin Receptor Substrate family (IRS1-4). Once phosphorylated, IRS is able to bind to other proteins, the most important of which discovered so far is Type 1A phosphatidylinositol 3-kinase (PI 3-kinase). Insulin-stimulated glucose uptake is mediated by GLUT4 translocation to the cell surface and it is known that the complex formed by the association of IRS and PI 3-kinase is essential to this role (19). The intermediates between this activation and GLUT4 translocation are not entirely clear but appear to involve Protein Kinase B (PKB) and isoforms of Protein Kinase C (PKC). Experiments have shown that expression of PKB and PKC within certain cells promotes GLUT4 translocation and blocking the function of these kinases inhibits this GLUT4 translocation (20) (21).

Pathways independent of PI 3-kinase

The pathways, however, are not as straight forward as outlined above. Other influences, not fully understood at present, are also interacting with this system. For example, reduced PI 3-kinase activation does not always lead to attenuation of downstream signalling by PKC and PKB, as would be expected (22). There is much redundancy built into the network such that only partial PI 3-kinase activation is needed to fully initiate downstream signalling.

PI 3-kinase activation can be promoted by other means and Interleukin-4 (IL-4) is a cytokine that can do this. However, PI 3-kinase activated by this means fails to augment GLUT4 translocation and this suggests that additional signalling pathways must be present (23). This is also insinuated by the data showing that naturally occurring mutant insulin receptors which still activate PI 3-kinase fail to influence GLUT4 translocation (24). There is also evidence that PI 3-kinase independent mechanisms exist for GLUT4 translocation in that cells treated with wortmannin (a PI 3-kinase inhibitor) can still exhibit enhanced GLUT4 translocation when treated with insulin and phosphatidylinositol triphosphate (PI(3,4,5)P3). This effect is not seen in PI(3,4,5)P3-treated cells alone nor in combination with wortmannin (25).

A pathway independent of PI 3-kinase has been proposed on the basis of experiments showing that the tyrosine phosphorylated InR can cause tyrosine phosphorylation of a cellular protein termed 'Cbl' in addition to IRS. This Cbl phosphorylation occurs in the presence of a facilitating protein, Cbl adapter protein (CAP) (26), and non-functional mutations of CAP inhibit GLUT4 translocation (20). This pathway is thought to function in parallel with the PI 3-kinase system and it appears to be involved in the interactions of GLUT4 vesicles and the cell membrane.

Lipid Rafts and Intracellular GLUT4 Trafficking

The cell membrane is composed of a phospholipid bi-layer but has specialised areas with distinct lipid and protein structures. Such areas are termed lipid raft microdomains and are thought to facilitate specialised functions (27). Such lipid rafts are thought to be important in the exocytosis of intracellular vesicles containing the GLUT4 transporter especially those microdomains containing the protein caveolin. Experimental disruption of these lipid rafts by cellular expression of dominant caveolin mutant protein results in inhibition of insulin-mediated GLUT4 translocation (28). The phosphorylated cbl protein is known to migrate intracellularly towards lipid raft domains rich in the protein flotillin and this process is thought to be dependant on CAP (29). Here it is thought to recruit a protein complex termed CrkII-C3G. The C3G component is thought to be vital in activating a further protein named TC10, which is a small GTP-binding protein (30). There is experimental evidence that mutant forms of TC10 also have an inhibitory influence on the translocation of GLUT4 in response to insulin (28).

These data all point towards a parallel signalling system working independently of PI 3-kinase and although PI 3-kinase activation is a prerequisite for GLUT4 translocation, successful enhancement of this translocation requires this parallel system involving CAP-Cbl, TC10 and the lipid raft to be intact.

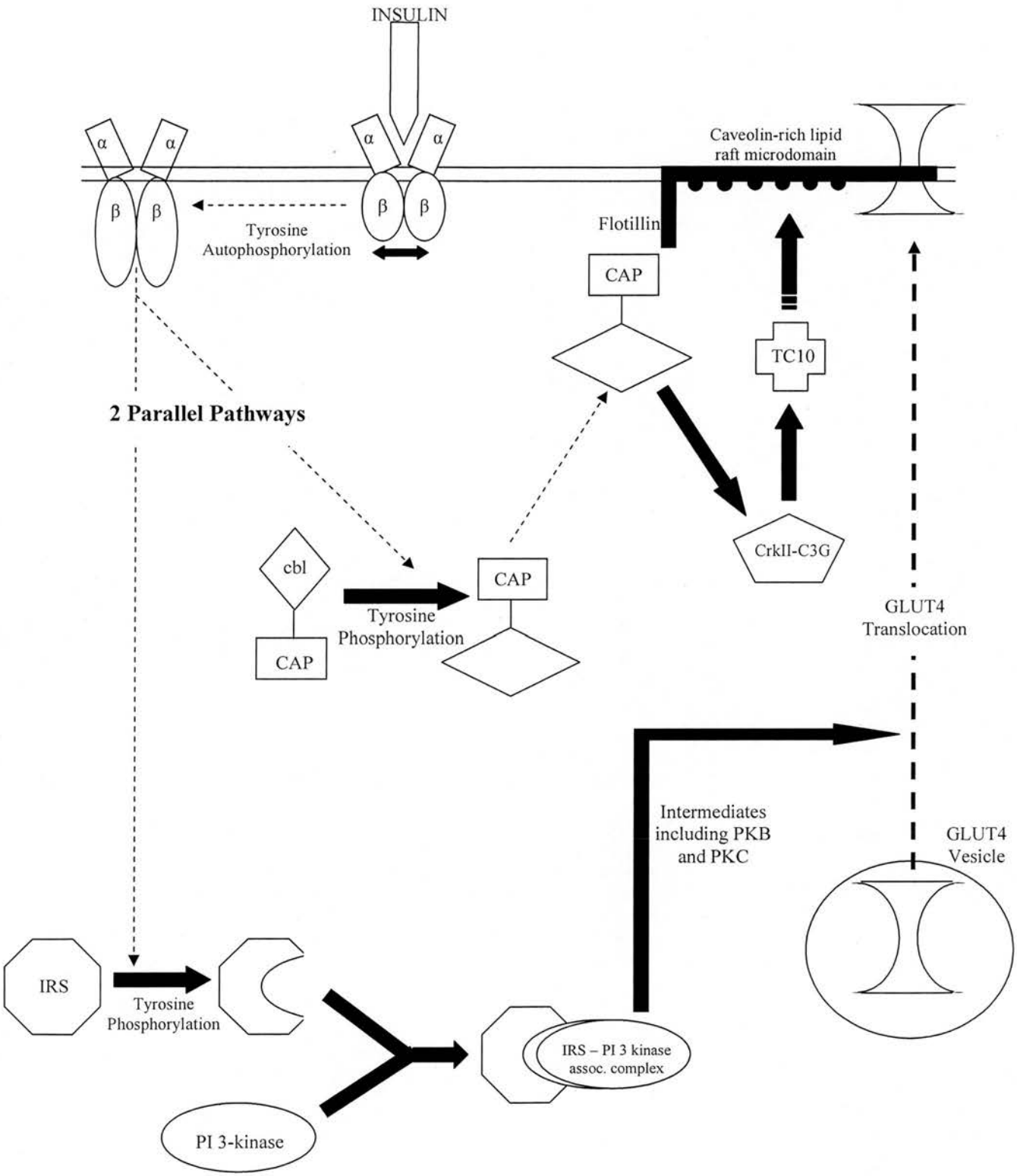
Down-regulation of Insulin Signalling

There are also down-regulating pathways operating at different levels. It is known that if the InR or IRS-1 is subjected to phosphorylation of serine or threonine residues in place of tyrosine, their function is attenuated. InR with serine/threonine phosphorylation exhibits less tyrosine kinase activity and IRS-1 is does not interact with PI 3-kinase as a consequence of serine/threonine phosphorylation. A number of enzymes that catalyse serine/threonine phosphorylation are known to exist. These include mitogen-activated protein kinase (MAP Kinase), atypical protein kinase C (PKC) and several others but their exact role in-vivo is not known (20). There is also

a family of enzymes identified termed protein tyrosine phosphatases (PTPases), which can de-phosphorylate the InR thus reducing its tyrosine kinase activity and attenuating insulin signalling. High levels of PTP1B (a specific PTPase) have been described in some insulin resistant humans. Information on its exact in-vivo role is lacking (31).

Increasing amounts of data are becoming available about the intricacies of intracellular insulin signalling and an increasing number of molecules and their roles within the system are being discovered on a month to month basis. This is an area which is growing rapidly and it is likely that our understanding of the signalling network is will evolve considerably in the forth-coming years. Figure 1.4 below summarises some of the key steps and molecules involved in this complex system.

Figure 1.4: Schematic showing some of the known intra-cellular pathways involved in insulin signalling



Mechanisms of Insulin Resistance – Potential Targets

Genetic Mutations

The network propagating and regulating insulin signalling is intricate. Potentially, any genetic mutation that influences the expression, availability or function of any polypeptide within this network, could affect insulin signalling. Animal models have proved useful in studies and data are relatively lacking in human models of disease. As each polypeptide within the network is coded on 2 chromosomes, an individual can be heterozygous or homozygous for any given mutation. In general, heterozygous mutations tend to be phenotypically almost normal, in that because of the degree of redundancy built in to the system, enough functional polypeptide is usually made available from the one functioning allele. For phenotypic differences to manifest, there needs either to be homozygous mutations or more commonly, compound heterozygous mutation (different mutations affecting the same allele). Phenotypic insulin resistance is probably most common in those with heterozygous mutations affecting several proteins within the network, leading to a polygenic model of insulin resistance. However, it possible for a heterozygous single allele mutation to produce significant insulin resistance. In theory, a mutated polypeptide could interfere with the function of the normally-expressed dominant polypeptide and therefore create a much greater defect in insulin signalling than would otherwise be expected.

Clinical insulin resistance caused exclusively by mutations affecting the insulin receptor itself are very rare, but have been described in humans. Mutations are able to negatively influence InR expression, insulin-binding or InR tyrosine kinase activity and result in a variety of clinical syndromes in humans (32). In animal models, mice with homozygous InR knockout exhibit severe insulin resistance and do not live long (33). However, those who are heterozygous have no measurable insulin resistance at all – a pointer to the built-in redundancy. This is also true of IRS-1 with homozygous knockout mice being insulin resistant but heterozygotes showing no significant defect in insulin signalling (34). However, mice heterozygous for both the InR and IRS-1 knockout develop insulin resistance and diabetes in a multi-hit polygenic fashion (35).

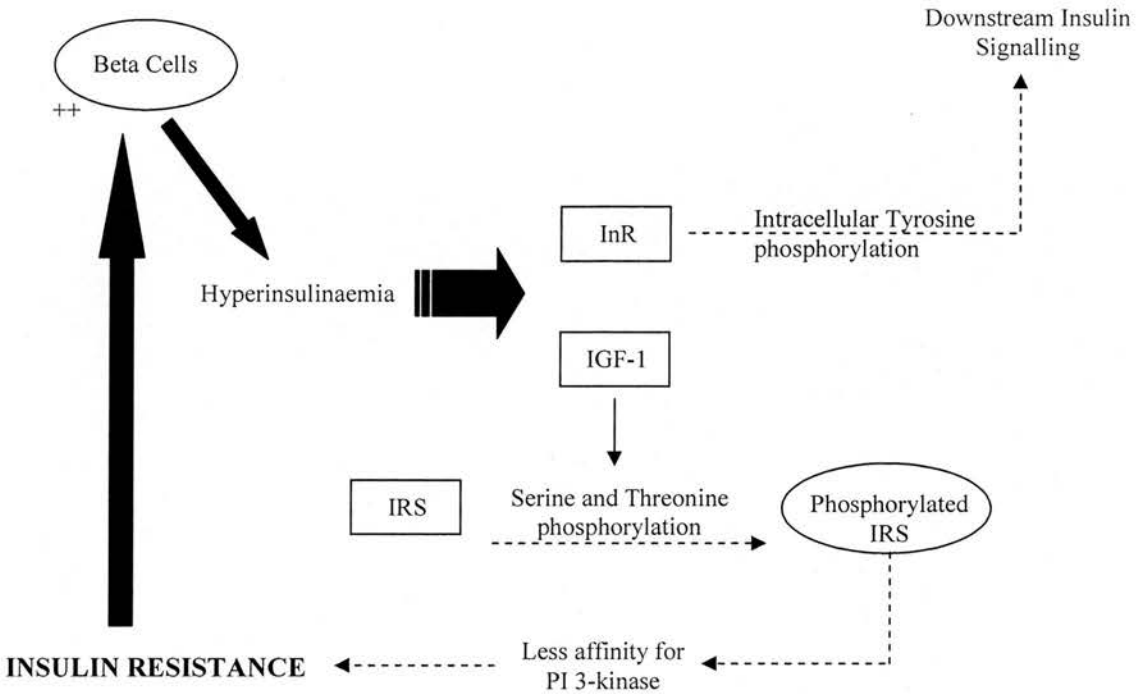
It is likely that these principles apply in humans at least to some extent, with the majority of inherited insulin resistance being mediated in this polygenic multi-hit manner. Mutations can improve insulin sensitivity as well, at least in mice. PTPases attenuate insulin signalling by de-phosphorylation on tyrosine residues and PTP1B knockout mice are known to exhibit enhanced insulin sensitivity and less obesity (36). These specific examples studied in animal models shed some light onto potential mechanisms. Every polypeptide within the network of insulin signalling is vulnerable to mutation and this creates a huge possible substrate on which genetic mutations are potentially capable of modifying insulin signalling and influencing clinical parameters of insulin sensitivity.

Hyperinsulinaemia

Reduced peripheral insulin sensitivity, caused by whatever means, results in an increase in the beta islet cell activity initially, as a compensatory mechanism and leading to hyperinsulinaemia, which itself is used as an index of insulin resistance. However, there is evidence to suggest that chronic hyperinsulinaemia has detrimental effects on insulin sensitivity in the longer term. It is known that long-term exposure of cells to insulin results in a dampening of insulin signalling which is mediated by serine and threonine phosphorylation of IRS-1 (21) . This in turn attenuates the ability of IRS-1 to associate with PI 3-kinase as well as reducing it tendency to be phosphorylated on tyrosine residues by the activated InR.

The exact mechanism by which this serine/threonine phosphorylation takes place is poorly understood but several protein kinases capable of serine/threonine phosphorylation have been identified and furthermore inhibition of protein kinase C (one such enzyme) has been shown to reduce insulin resistance (37). It has been hypothesised that Insulin-like Growth Factor (IGF) receptors, which can be activated by high concentrations of insulin, may be responsible for the activation of intracellular protein kinases which subsequently phosphorylate IRS-1 and InR on serine and threonine residues, thereby attenuating insulin signalling (20). If a feedback circuit like this does exist, then potentially a vicious cycle of insulin resistance may a play a part in the amplification of insulin resistance as shown in figure 5.

Figure 1.5: Schematic showing how hyperinsulinaemia could potentially be involved in the pathogenesis of insulin resistance



Inflammation

It has been known from animal models for some time that high dose salicylate has beneficial effects on insulin resistance and glucose tolerance (38).With an anti-inflammatory effect leading to enhanced insulin sensitivity, it is reasonable to infer that pro-inflammatory pathways and cytokines may interact with insulin signalling. Weight is added to this by the observation that the pro-inflammatory cytokine Tumour Necrosis Factor alpha (TNF-α) is increased in adipose tissue of obese insulin resistant subjects. Furthermore, it is known that TNF-α is an inhibitor of tyrosine phosphorylation of IRS-1 and InR and, in animal models, neutralisation of TNF-α improves insulin sensitivity by increased tyrosine phosphorylation of InR (39). This effect of TNF-α is thought to be due to its promotion of serine and threonine phosphorylation of IRS-1 (40) with subsequent dampening of insulin signalling, thereby potentiating insulin resistance. The interaction of the many other pro-inflammatory cytokines with insulin signalling remains obscure.

Adipose Tissue and Lipids

The insulin resistance syndrome includes dyslipidaemia and central adiposity as central features. The dyslipidaemia encompasses hypertriglyceridaemia, high levels of LDL-cholesterol, low levels of HDL-cholesterol and high circulating levels of non-esterified fatty acids (NEFAs). It is known that obese individuals are more insulin resistant compared to lean subjects (41). Furthermore, the distribution of adipose tissue appears crucial with central visceral deposits correlating closely with insulin resistance. Although the exact mechanism of this association is unclear, it has been suggested that visceral adipocytes are more sensitive to catecholamine-induced lipolysis and less sensitive to insulin-mediated anti-lipolytic stimuli (42). This would tend to increase whole-body rates of lipolysis and thus levels of circulating NEFAs which are able to render peripheral tissues insulin resistant by mechanisms discussed below.

Skeletal Muscle

Most recently, the lipid content of skeletal muscle has been studied and several groups have found that levels of insulin resistance are correlated with intracellular lipid within myocytes (intramyocellular lipid – IMCL). This has been demonstrated non-invasively by proton magnetic resonance spectroscopy and shown to be a valid association not only in obese individuals independent of whole body mass (43), but also in lean insulin resistant offspring of patients with type 2 diabetes (44). Studies looking at weight loss and the effects of IMCL depletion specifically on whole body indices of insulin resistance have found that improvements in insulin sensitivity correlate with the loss of IMCL, suggesting that IMCL may have a central aetiological role in systemic insulin resistance (45). The mechanism by which this potentially occurs is not known.

Non-esterified Fatty Acids

It has been known for some time that insulin resistance is almost invariably associated with high circulating levels of NEFAs. Whether this is merely an association, or the cause or effect of insulin resistance, has been the subject of debate. Insulin promotes glucose transport into cells by its effect on the cell membrane glucose transporter

protein GLUT4. This occurs in many cell types including adipose tissue. Glucose is essential in adipocytes for the process of triglyceride formation in which glycerol-phosphate (formed from glucose) is esterified with fatty acid chains. The cycle of triglyceride lipolysis and esterification goes on constantly within adipocytes and the enzymes involved are insulin dependant. Resistance to insulin in adipose tissue pushes the direction of the cycle in favour of lipolysis as well as reducing glucose transport into adipocytes affecting the availability of glycerol-phosphate, needed for fatty acid esterification. Insulin resistance can therefore potentially promote increased levels of circulating NEFAs by this method.

Conversely, it is known that high concentrations of NEFAs can promote resistance to insulin-mediated glucose uptake. This has been demonstrated during lipid infusions which raise serum NEFAs, by reducing whole body glucose disposal during hyperinsulinaemic, euglycaemic conditions (46). The mechanism by which this occurs is at least in part due to substrate competition described by Randle in 1963 in which relatively high levels of fatty acid oxidation are postulated to inactivate pyruvate dehydrogenase leading to intracellular glucose-6-phosphate accumulation and ultimately a reduction in glucose uptake. (47). More recently, a non-oxidative mechanism has been described in which high concentrations of NEFAs not only reduce glucose disposal and oxidation under hyperinsulinaemic conditions, but also significantly attenuate the expected increases in glucose-6-phosphate and glycogen in calf muscle. These in-vivo data were collected using nuclear magnetic resonance spectroscopy. The observed attenuation in muscle glucose-6-phosphate by high levels of fatty acids, which is contrary to what the Randle Hypothesis would predict, tends to suggest that the defect lies at the level of glucose transport or phosphorylation (48).

The cellular mechanism by which NEFAs potentially attenuate glucose transport as suggested by Roden et al (48) has been investigated. It is known that insulin signalling is dampened in the presence of high NEFA concentrations. This is manifest by reduced activation of the IRS – P13-kinase complex in response to insulin under these conditions (49). Ultimately this will lead to a reduction in GLUT4 translocation resulting in decreased glucose uptake.

Obesity

Obesity characterised by adipocyte hyperplasia and hypertrophy, has a complex relationship with insulin resistance. Being overweight is certainly associated with insulin resistance and this is likely to be at least in part mediated by higher circulating levels of NEFA. However, there are mechanisms by which NEFA promote insulin resistance and potentially a positive feed-back loop in which levels of insulin resistance could be amplified exists.

Summary

There are several different mechanisms by which insulin resistance may be propagated with genetic influences forming a background on which environmental influences such as body fat distribution and systemic inflammation can interact. However, it is clear that these influences do not act in isolation and it is likely that they work together, with even the possibility of positive feed-back and amplification once the processes to promote insulin resistance have been initiated.

Insulin Resistance and Atheromatous Disease

Coronary Angiographic Data

Several studies have looked at patients who have undergone cardiac catheterisation and have found links between markers of insulin resistance and atheromatous disease. Tsuchihashi et al, looking at 95 non-diabetic subjects with abnormal coronary angiography, showed that 24% had insulin resistance by their criteria (serum insulin >= 60.4 IU/L 120mins after 75g oral glucose load). Levels of insulin resistance showed a close linear relationship to the stenosis score, suggesting that the degree of insulin resistance is correlated to the severity of the atheromatous disease (50). Takezako et al looked at a group of 66 subjects with abnormal coronary angiograms and divided them into 3 tertiles according to their Gensini Score of coronary artery disease severity. They found that patients in the highest tertile were more insulin resistant (51). Shinozaki et al showed that patients with angiographically documented coronary artery disease were significantly more insulin resistant than their counter-parts with no coronary artery disease. Severity of atheromatous disease was correlated well with insulin resistance (52).

These data suggests that insulin resistance in itself is a risk factor for coronary atheroma. This is independent of other factors within the insulin resistance syndrome as no differences among subgroups examined for lipids, hypertension and uric acid were found (50;51;53) The level of insulin resistance is related to the severity of atheromatous disease observed in these studies.

Non-invasive Ultrasound Data

In addition to data from coronary angiograms, there are several studies, which have looked at non-invasive ultrasound data of the common or internal carotid artery. Investigators have measured intima-medial thickness, which is a guide to the degree of atheroma in a vessel. The Insulin Resistance Atherosclerosis Study (IRAS) investigators showed that the minimal model index of insulin resistance was positively correlated with carotid intima-medial thickness (IMT) in both Hispanics and non-Hispanic whites, but not in the Black population. The association is stronger in the internal carotid rather than the common carotid artery (54). They demonstrated approximately a 30μm decrease in IMT with every 1MU increase in insulin sensitivity in the white Hispanic and non-Hispanic populations. The effect was attenuated by approximately 20% when corrected for other cardiovascular risk factors but remained independently significant.

Shinozaki et al, looked at a population with normal coronaries angiographically (a mixture of patients with vasospastic angina and controls) with similar findings. Common carotid intima-media thickness was correlated to insulin resistance in both groups (55). More recent data from Hedblad et al showed that although an insulin resistant population had a greater IMT, the association between IMT and insulin resistance was obliterated when other features of the insulin resistance syndrome were taken into account (most notably hypertension) (56). These studies tend to suggest that insulin resistance is a risk factor for atheroma in general, although independently this effect is likely to be modest at best.

These invasive angiographic and non-invasive ultrasound data are summarised in table 1.3 overleaf.

Table 1.3: Cross-sectional studies looking at markers of IR and general and coronary atheroma

Study Name	Number in Study	Measure of Insulin Resistance (IR)	Marker of Atheroma	Results / Comments
Tsuchihashi et al (50)	95 non-diabetic	120 min post glucose-load insulin	Coronary Angiogram	Linear correlation between stenosis score and IR ($r=0.266$, $p=0.009$. Significant differences in coronary stenosis and calcification score between those with 120min insulin < and > 60.4IU/L.
Takezako et al (51)	66 patients with coronary disease	OGTT and HOMA index	Coronary Angiogram Gensini's score	Patients in top tertile of Gensini Score were more insulin resistant compared to those in bottom tertile in terms of fasting insulin, 120min insulin and HOMA-IR
Shinozaki et al (52)	38 lean non-diabetic subjects with varying coronary disease and glucose tolerance	Steady state plasma glucose (SSPG)	Coronary Angiogram with modified Gensini score	Those with coronary disease more IR than those without both in normal and impaired glucose tolerance groups. Coronary atherosclerosis score correlated to 2 hour insulin area in subjects with normal glucose tolerance ($r=0.78$, $p<0.05$)
IRAS investigators (54)	1397 individuals of varying ethnicity	Minimal model	Ultrasound thickness of internal and common carotid intima-media (IMT)	Negative assoc between IMT and insulin sensitivity in Hispanics and white population (not in Black population). 30μm decrease in internal carotid IMT with every 1MU increase in insulin sensitivity (attenuated by 20% when adjusted for cardiovascular risk factors)
Shinozaki et al (55)	64 subjects with normal coronaries angiographically. (24 controls and 40 with vasospastic angina)	2 hour OGTT (area under insulin curve)	Ultrasound thickness of common carotid intima-media thickness	Correlation seen between IMT and 2 hour insulin area in patients with vasospastic angina ($r=0.45$, $p<0.01$)
Hedblad et al (56)	4816 non-diabetic subjects without symptomatic coronary disease	HOMA model with top quartile categorised as IR	Ultrasound thickness of common carotid intima-media thickness	Significant difference in IMT between IR and non-IR subjects. (0.78mm Vs 0.754mm – $p<0.001$) HOMA-IR index assoc with IMT but not after correction for other features of IR syndrome

Insulin Resistance and Coronary Heart Disease

The preceding section has highlighted the association between insulin resistance and atheromatous disease. This link does translate into clinical outcomes, as shown in many prospective trials looking at insulin resistant subjects free from coronary heart disease (CHD) at baseline and following them up for cardiac events. Because of the large numbers involved in these large epidemiological studies, indices of insulin resistance based on fasting serum insulin were most commonly used. Several studies indicate that insulin resistant subjects are more likely to develop coronary heart disease than their insulin-sensitive counter-parts. There has been some controversy as to whether the increased risk applies to men, or women or both groups. These studies have been summarised in table 1.4

The ARIC investigators looked at a 13446 strong cohort of patients with and without diabetes, and followed them up for 4-7 years. They were able to demonstrate that non-diabetic women in the highest quintile of insulin concentration at baseline (thus highest levels of insulin resistance) had a multi-variable adjusted relative risk of the development of coronary heart disease of 2.82, compared to women in the lowest quintile of insulin concentration. However, this same trend was not observed in the non-diabetic men within this cohort. As expected, subjects with diabetes, and therefore the highest levels of insulin resistance, showed a significant trend for the development of coronary heart disease. A relative risk of 3.45 and 2.52 was conferred upon women and men respectively, compared with non-diabetic subjects irrespective of their levels of insulin resistance (57).

In contrast to the ARIC investigators, The Quebec Cardiovascular Society Study did find a prospective correlation between baseline insulin-resistance and CHD in men. Depres et al collected data from 2103 men and followed them for 5 years. The 91 non-diabetic men in this group who developed ischaemic heart disease (IHD) were matched with 105 controls within this cohort and were found to have baseline insulin concentrations 18% higher than them. When corrected for other cardiovascular risks, the link between insulin levels and IHD remained significant, with an odds ratio of 1.6

(1.1-2.3) for the risk of IHD with every 1 standard deviation increase in insulin level within this population (58).

The 9½ year data from the Helsinki Policeman Study also supported the concept that plasma insulin levels correlate well with CHD risk. 982 men were followed up prospectively and higher insulin levels were associated, independently of other risk factors, with the development of CHD (59). There was a non-linear relationship, with the subjects in the top decile of insulin having the highest rate of development of CHD events. In the 22 year follow-up data, the measure of insulin resistance used was area under the curve (AUC) of insulin response. This was grouped together with other factors relating to the insulin resistance syndrome including AUC glucose, triglyceride levels, mean blood pressure and body mass index (BMI), to produce a single insulin resistance factor. This single factor, although not strictly a measure of insulin resistance on its own, was correlated positively with risk of both CHD (age-adjusted hazard ratio 1.28) and stroke (age-adjusted hazard ratio 1.64) (60).

Table 1.4: summary of prospective trials looking at insulin resistance and coronary heart disease

Study Name	Number in Study	Follow-up Period	Measure of Insulin Resistance	IR related to CHD in men	IR related to CHD in women	Comments
ARIC (57)	13446 men + women (diabetic included) Middle-aged population	4-7 years	Fasting insulin	no	yes	Diabetics had greater incidence CHD Linear trend for quintiles of fasting insulin and CHD incidence
Quebec City (58)	2103 men (45-76 yrs)	5 years	Fasting insulin	yes	-	Fasting insulin levels 18% higher in men who develop CHD compared with matched controls who did not, despite correcting for other risk factors
Helsinki Policeman (59)	982 men (35-64 yrs)	9 ½ years	Insulin levels related to 2 hour OGTT	yes	-	Highest incidence CHD in top decile of plasma insulin levels
Helsinki Policeman (60)	970 men (35-64 yrs)	22 years	Insulin Resistance Factor * (IRF)	yes	-	Age-adjusted hazard ratio for IRF* was 1.28 with regard to CHD risk and 1.64 with regard to stroke risk
Paris Prospective Study (61)	7164 men (43-54 yrs)	11.5 years	Fasting insulin	yes	-	Fasting plasma insulin positively associated with risk of CHD death independently of other risk factors
Paris Prospective Study (62)	6903 men (43-54 yrs)	15 years	2 hour post-glucose insulin level	yes	-	Fasting insulin not an independent predictor of CHD death. 2 hr post-glucose load insulin levels in highest quintile, are independent predictors of CHD death
Barilla factory (63)	647 men + women Middle-aged population	15 years	Post-glucose insulin	yes	yes	Those in highest quintile of insulin response had 3-fold increased risk of CHD
Eastern Finland (64)	1069 men + women (65-74yrs)	7 years	Insulin Resistance Factor** (IRF)	yes	no	IRF** predicted CHD events in men but not women during follow-up
British Regional Heart Study (65)	5500 men (40-69yrs)	11.5 years	Random insulin levels	yes	-	Men in highest decile of insulin levels had 2-fold increase in CHD events compared with men in other 9 deciles

Danish Study (66)	1052 40 year-old men and women	17 years	Fasting insulin	yes	yes	Significant independent association between fasting insulin and CHD
Australian Study (67)	2971 men + women aged > 20years	23 years	I hour post-glucose load insulin levels	yes	no	Highest and lowest quintiles of insulin associated with CHD deaths in men.
South Wales (68)	2512 men (45-59 yrs)	5 years	Fasting insulin	no	-	Men in top 20% of insulin levels had increased CHD in univariate analysis. Significance was lost when corrected for other risk factors
Edinburgh Study (69)	107 men all 40 years-old	12 years	Post-glucose insulin levels	no	-	11 men developed CHD and their insulin levels were similar to those that remained CHD-free
Ducimetiere et al (70)	7246 men (43-54 yrs)	63 months	Fasting insulin	yes	-	Fasting insulin level independently associated with risk of CHD event. 2 hr post-glucose insulin level lost significance when corrected for other risk factors
Malmo Study (71)	4748 men and women (non-diabetic)	6 years	HOMA-IR (IR defined as > 75th percentile)	yes	yes	IR conferred a relative risk of 2.18 on coronary events and 1.62 on all-cause mortality even when adjusted for other cardiovascular risk factors.
TRACE Study Group (72)	494 non-diabetic patients with confirmed myocardial infarction	6-8 years	Fasting insulin (IR defined as in upper quartile)	yes	yes	Hyper-insulinaemia was the only significant variable after multi-variate analysis in predicting death (Relative risk 1.54, p=0.04)

* IR factor comprised BMI, Subscapular skinfold thickness, AUC Insulin, AUC glucose, mean blood pressure, triglycerides, maximal O(2) uptake

** IR factor reflected BMI, waist:hip ratio, triglycerides, fasting insulin and fasting glucose levels

The Paris Prospective Study 10 year follow-up data showed a similar trend with fasting plasma insulin being positively associated with annual risk of CHD mortality, after analysis by a multivariate Cox model, amongst the 7164 men studied after 10 years (61). However, in the 15-year follow-up of the non-diabetic subjects in this group, fasting insulin level ceased to be a significant independent predictor for CHD mortality, but a 2 hour post-glucose insulin levels above the 5^{th} quintile was a significant independent predictor of CHD death (62).

Several other long-term prospective trials, looking at insulin resistance and CHD risk, have been published and a summary of these is shown in table 3. As can be seen, there is much more data from studies in male subjects. 2 out of the 5 studies looking at women did not find an association between insulin resistance and CHD. Out of all the studies looking at men only 3 out of 14 did not find a similar association.

The large number of prospective studies provide good evidence, and it is now generally accepted that insulin-resistance is at least a modest independent cardiovascular risk factor, with much more evidence existing for men than women. Insulin resistance rarely occurs on its own, without other cardiovascular risk factors, but even correcting for these, insulin resistance has its place as an independent risk factor. A feature that emerged in most of the prospective data is the non-linear relationship between levels of insulin resistance and CHD risk. Several studies divided individuals into quartiles, quintiles and even deciles of insulin resistance. Such studies showed a significant CHD risk in those in the top division compared to all others, or sometimes just those in the bottom division (57;59;62;63;65;67;71;72). This statistical trend suggests that only individuals with the highest levels of insulin resistance are at increased risk of CHD.

A Meta-analysis by Ruige et al in 1997 looked at the available evidence linking insulin levels (as a marker of insulin resistance) with rates of cardiovascular event including myocardial infarction, coronary death and ECG abnormalities. A total of 17 studies were identified by authors with 12 providing enough data to calculate an

overall relative risk. The authors found that hyperinsulinaemia is a weak independent predictor of cardiovascular disease with a relative risk of 1.18 conferred on those at the 75th percentile of serum insulin compared to those at the 25th percentile (95% confidence intervals 1.08-1.29) (11). However, given the non-linear association already alluded to, this relative risk is likely to be greater in magnitude if the 90th or 95th percentile is compared to the 25th percentile.

It has been known for some time that the cardiovascular risk of individuals with type 2 diabetes is higher than those without. The primary risk of a diabetic individual is equivalent to the risk of a non-diabetic individual who has had a coronary event (73). Patients with type 2 diabetes are by definition all insulin resistant, but the level of resistance varies to some degree. Inchiostro et al showed that even amongst individuals with diabetes, indices of insulin resistance are significantly higher in those patients with IHD compared to those without. Total cholesterol and hypertension were also important correlates. However, after correction for age and duration of diabetes, only insulin sensitivity was correlated to the age of onset of IHD (74). This would tend to suggest that insulin resistance, itself, is the important factor in conferring cardiovascular risk in patients with type 2 diabetes.

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CHAPTER 2

Cardiac Syndrome X –

Myocardial Ischaemia, Insulin Resistance and Vascular Dysfunction

Myocardial Ischaemia and Vasomotor Dysfunction

Traditionally, myocardial ischaemia is synonymous with coronary artery disease in which lumen-narrowing atheromatous plaques limit myocardial blood flow during times of increased metabolic demand, resulting in myocardial lactate production which is thought to be responsible for the production of angina pectoris. However, coronary angiography, which identifies diseased coronary arteries, is an anatomical investigation. Although it provides information about the coronary circulation, which if diseased, may correlate with the potential for ischaemia, is does not provide any direct physiological measure of myocardial ischaemia.

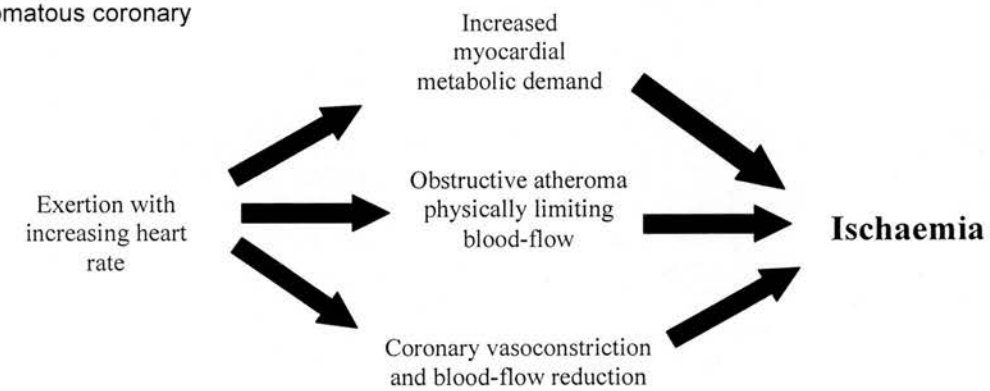
Vasomotor Abnormalities in Atheromatous Coronaries

There is evidence that myocardial ischaemia can at least in part be due to functional abnormalities of vascular function. During times of increased myocardial metabolic demand, coronary artery and microvascular vasodilation takes place by means of physiological autoregulation. This process can be impaired leading to a potential mechanism of impaired vasodilator reserve and thus ischaemia. These abnormal vasomotor responses are known to occur in atheromatous coronary arteries. For example Gage et al have demonstrated that patients with coronary artery disease develop coronary vasoconstriction in stenotic coronary arteries in response to bicycle exercise during coronary angiography. This is in contrast to normal coronary arteries (in the same group of patients) in which vasodilation was observed during exercise. Administration of intra-coronary nitrate abolished this vasocontrictive effect and resulted in less evidence of ischaemia (1). This suggests that it is likely to be a clinically important phenomenon. Similar finding were presented by Gordon et al again showing that diseased coronary segments are much more likely to undergo vasoconstriction in response to bicycle exercise during angiography. Paradoxically, intra-coronary acetyl choline, which produced vasodilation in the smooth coronary segments, caused vasoconstriction in a significant number of atheromatous segments

(2). The same sort of paradoxical vasocontrictive response has been reported in patients undergoing angiography with atrial-pacing induced tachycardia. Those patients with smooth coronaries tended towards coronary vasodilation with a resultant increase in coronary blood flow as assessed by Doppler velocity recordings. This contrasts with the response in those with significant coronary atheromatous disease, in whom vasoconstriction and a reduction in coronary blood flow was recorded (3).

These three studies all point to the same phenomenon in which atheromatous coronary artery segments tend to paradoxically vasoconstrict or at least fail to vasodilate normally during times of increased myocardial demand. This leads to a demonstrable reduction in coronary blood flow and results in exacerbation of ischaemia in a myocardial substrate already experiencing higher metabolic demand, as illustrated below in figure 2.1

Fig 2.1
Mechanisms of ischaemia in those with atheromatous coronary



To prove this abnormal vasomotor response has an independent role in myocardial ischaemia is difficult, because of co-existing flow-limiting atheromatous plaques which provide a physical obstruction and are thus capable of producing ischaemia alone.

However, there exists a group of patients with smooth unobstructed coronary arteries in whom there is evidence of myocardial ischaemia as manifest clinically by typical symptoms and the results of non-invasive investigations. This is the patient group termed as "Syndrome X" and at least a subset of these patients will have microvascular angina, which is thought to be due to dysfunctional vasomotor responses in the coronary microcirculation. This group of patients afford us the opportunity to study functional vessel abnormalities without the influence of co-existing obstructive atheromatous disease.

Cardiac Syndrome X and Microvascular Angina

There is a well recognised clinical syndrome in which inducible myocardial ischaemia can manifest despite angiographically undiseased coronary arteries. Indeed, it has been reported that up to 30% of diagnostic angiograms identify normal coronary arteries in some series. The term "Cardiac Syndrome X" was first used by Kemp in 1973 to describe this patient group (4). The 3 essential clinical features that Kemp described were:

- Typical anginal-type chest pain
- Unobstructed coronary arteries angiographically
- Positive exercise stress test

In the absence of clinical or angiographic evidence of coronary artery spasm.

In practice, patients who fulfil these criteria form a large heterogeneous group encompassing those with genuine myocardial ischaemia as well as a probably larger proportion with non-cardiac pain. The reason for this mixtures lies with the lack of an accessible 'gold-standard' method for the demonstration of ischaemia. The stress ECG is the most widely used tool for assessment of myocardial ischaemia in clinical practice. However, the exercise tolerance test (ETT) is a relatively non-specific test (as well as relatively insensitive) which requires subjective interpretation even in patients with coronary disease (5). These features mean that is not uncommon for patients with non-cardiac pain to have 'false positive' ETTs and therefore be included under the label of "Syndrome X".

Patient Characteristics

Female Predominance

A significant number of diagnostic coronary angiograms demonstrate no significant obstructive disease – up to 30% in some reports. The series of 99 patients followed-up by Kaski et al is fairly typical of those reported in the literature although their patients fulfilling the 'Syndrome X' criteria had a particularly stringent emphasis on there being at least 1mm of ST depression on an exercise ECG. They found a female bias of almost 80% with almost 62% of the 78 women being postmenopausal. 35 of these women (45%) had a hysterectomy performed an average of 8 years before the onset of chest pain (6).This female dominance has been reported in previous studies (7;8). In series in which this female dominance was not apparent the ECG criteria tended not to be applied so rigorously (9-11) and potentially therefore many more subjects with non-cardiac pain were included.

Hypertension

Although patients with hypertension were excluded from the series by Kaski et al, only 11 out of 138 (8%) patients initially screened had a blood pressure >140/90mmHg. Eight of the 99 patients followed-up developed hypertension. Rates of hypertension therefore seem comparable with the general population. Most patients had a normal resting ECG (81%) with the remainder having T wave abnormalities or minor ST segment changes and ambulatory ECG monitoring demonstrated transient ST-segment depression in 64 patient (65%). About half of these episodes were associated with symptoms of chest pain and 78% with a preceding tachycardia (6).

Metabolic perturbances especially high indices of insulin resistance have been reported in this group. This may have relevance to the pathophysiology and will be expanded upon below.

Coronary Angiography

Coronary angiography yields information about the coronary artery lumen and can exclude obstructive disease but this does not necessarily rule out 'sub-angiographic' atheroma. It has been shown that a significant proportion of patients deemed to have 'normal coronaries' in fact have non-lumen encroaching plaques. This proportion was as high as almost half in a group of 44 patients with previously normal coronaries and chest pain (12). A smaller series reported by Cox et al demonstrated sub-angiographic atheroma in 6 out 9 patients with normal coronaries undergoing repeat angiography because of unstable anginal symptoms (13). Wiedermann et all looked at 30 patients with cardiac 'Syndrome X' and showed that although all subjects had smooth unobstructed coronary arteries angiographically, only 12 (40%) had coronary arteries that were deemed normal by intra-vascular ultrasound. The remainder of the patients had either sub-angiographic atheromatous plaques (10 patients) or intimal thickening (8 patients) (14).

Prognosis

It had long been thought that having angiographically normal coronary arteries confers a benign long-term prognosis. For example, Kaski et al reported no cardiac deaths or myocardial infarcts in a cohort of 99 such patients with an average seven year follow-up (6). Kemp et al looked at 3136 patients with normal coronary angiograms and found a 7 year survival rate of 96%. This did drop to 92% for the 915 patients with mild, non-obstructive disease (15). However, more recent data suggest that these patients do have adverse cardiovascular outcomes especially if risk factors are present (16).

Foussas et al reported one sudden cardiac death and two more non-fatal myocardial infarctions in a series of 160 patients (1.9% event rate) with normal coronary arteries, during a mean follow-up period of 2.5 years (9). Similarly Isner et al reported 3 sudden deaths and 4 non-fatal myocardial infarcts a group of 121 patients (5.8% event rate) with the same characteristics after mean follow-up of over 4 years (7). Lichtlen et al followed 176 patients with normal coronaries for an average of over 12 years and

found similar rates of adverse events with 14 myocardial infarcts and 2 cardiac deaths (9.1% event rate) (17). Most recently, data published by the Women's Ischaemia Syndrome Evaluation (WISE) group showed that women with normal coronary arteries but non-invasive evidence of myocardial ischaemia (on magnetic resonance spectroscopy) had rates of cardiovascular events similar to that of a control group with known coronary disease, and a much increased rate compared to a group with angiographically normal coronaries and no non-invasive evidence of ischaemia. However, there were no reported myocardial infarctions or deaths within all women with normal coronary arteries over the 3 year follow-up and this similarity in cardiovascular events between the group with coronary disease and the group with normal coronaries but non-invasive ischaemia, appears to have been driven by recurrent admission with angina and repeat coronary angiography (18).

A very small proportion of patients with angiographically normal coronary arteries go on to develop significant coronary atheromatous disease (19). Cox et al looked at 138 consecutive patients with normal or almost normal coronaries. 24 patients during a five year follow-up required repeat angiography for ongoing symptoms. Only 2 of these had progressed to significant levels of coronary obstruction and these were patients with minimal disease at baseline with multiple cardiac risk factors and left bundle branch block on ECG (20).

Overall it appears that the cardiac risk of patients with normal coronaries is not as benign as once thought. Several series have shown that some of these patients do go on to have adverse cardiovascular events and in any case, the ongoing burden of symptoms requiring hospital admissions and even repeat coronary angiography is very significant. Those that do go on to have an adverse event tend to do so several years after their angiogram and often have a higher cardiac risk-factor profile (17). However, the concept of myocardial infarction in normal coronaries does exist (21) presumably due to non-lumen encroaching unstable plaque rupture or vasomotor disturbance.

A subgroup of patients with 'Syndrome X' has a less good prognosis. Orpherk et al followed such a group and found that of the 19 in whom left ventricular function was reassessed after four years, those with constant or rate-dependent left bundle branch block (LBBB) demonstrated a significant deterioration in left ventricular ejection fraction (22). Some have suggested that these patients with LBBB are in fact a subgroup with early cardiomyopathy.

Summary

Although 'Syndrome X' encompasses a heterogeneous group, there is at least a subset who have myocardial ischaemia and the evidence for this is discussed below. Many series demonstrate a female dominance and a postmenopausal presentation. Most have a normal resting ECG and a significant proportion may have sub-angiographic coronary atheroma. Insulin resistance has been reported by several groups with the evidence presented later in this chapter. The long-term prognosis in these patients is generally not as good as previously thought and some patients do have adverse events. Studies looking at long-term prognosis are summarised in table 2.1 below. These patients tend to have a higher cardiac risk profile and therefore this may be related to sub-angiographic coronary atheroma.

Table 2.1: Summary of studies looking at prognosis in patients with normal coronary arteries

Study	Population	Characteristics	Mean/median follow-up	Outcomes
Kaski et al (6)	99 patients 80% female mean age 48.5 yrs	angina and normal CA Minimum of 1mm ST-segment depression on ETT	7 yrs	no MI or death 89% ongoing symptoms
Foussas et al (9)	160 patients 31% women	angina and normal CA 40% women with +ve ETT 10% men with +ve ETT	2.5 yrs	1 cardiac death 2 non-fatal MI 59% ongoing symptoms 41% on anti-anginal Rx
Isner et al (7)	121 patients 60% women mean age 49 yrs	angina and normal CA	4.3 yrs	3 with sudden death 4 non-fatal MI 80% ongoing symptoms 64% on anti-anginal Rx
Lichtlen et al (17)	176 patients 35% women mean age 48.3 yrs	angina and normal CA 18% with +ve ETT	12.4 yrs	2 cardiac deaths 12 non-fatal MI 81% ongoing symptoms
Kemp et al (15)	3136 patients	angina and normal CA	7 yrs	96% survival with normal CA
	915 patients	angina with mild coronary disease		92% survival with mild coronary disease
Opherk et al (22)	19 patients with repeat ECHO	angina and normal CA	4 yrs	Significant LV dysfunction developed in patients with constant and rate-induced LBBB but not in those with ST-segment depression.
WISE group (18)	74 women	angina and normal CA 14 women with abnormal NMR spect 60 women with normal NMR spect	3 yrs	no deaths/MI 36% and 21% rate of re-admission for angina and repeat angiography, respectively for women with abnormal NMR spect compared to 7% and 3% for normal NMR spect group
Halcox et al (23)	176 patients 22% women mean age 51.1 yrs	angina or abnormal non-invasive cardiac investigations and normal CA	3.8 yrs	2 cardiac deaths 5 non-fatal MI 5 admissions for angina

NMR Spect – nuclear magnetic resonance spectroscopy
LBBB – left bundle branch block
CA – coronary arteries

Evidence for Ischaemia in Cardiac 'Syndrome X'

Although some clinicians remain sceptical about the very existence of myocardial ischaemia with normal coronary arteries, several groups have demonstrated metabolic and indirect markers of myocardial ischaemia in subgroups of those labelled with 'Syndrome X'.

Myocardial Lactate Production

Resting myocardial substrate metabolism varies according to being in either the fasting or fed state. The fasting state tends to favour myocardial lipid uptake (mostly free fatty-acids) whereas post-prandially carbohydrate uptake dominates. During periods of increased myocardial workload, glucose uptake is enhanced with little change in free fatty-acid (FFA) uptake. Carbohydrate oxidation is therefore responsible for producing the majority of the 'extra' energy required under these circumstances. In an ischaemic myocardium, inadequate blood supply (and therefore oxygen) leads to the pyruvate formed during glycolysis not being oxidised, but converted to lactate by the action of lactate dehydrogenase (24). Reduced lactate extraction or even lactate production, reflected by coronary sinus lactate concentration > arterial lactate concentration, has therefore been used as a marker of myocardial ischaemia by several groups. It is perhaps the closest measure to a 'gold standard' for physiological demonstration of ischaemia that is available. Unfortunately, although it may be a fairly specific marker for ischaemia is it not particularly sensitive and will therefore miss a proportion of those with myocardial ischaemia (25).

Several groups have demonstrated lactate production during pacing experiments designed to mimic exertion, with an increased chronotropic response. This in itself is evidence for ischaemia in a subgroup of patients with 'Syndrome X'. Correlating ST-segment depression with lactate production in an individual patient is difficult. Boudoulas et al showed that as a group, those subjects with normal coronaries who

exhibited significant (2mm) ST segment depression to incremental pacing all produced lactate during pacing, although some lactate-producers did not show any ST-segment shift (26). This may be due to the relative lack of sensitivity of ST-segment change as a marker for ischaemia.

Mammohansingh et al added to these observations with 17 subjects all of whom had ST-segment depression during an exercise test despite normal coronaries. They found that although 14 out of 17 experienced chest pain during atrial pacing, only 4 exhibited abnormal lactate metabolism (27). The threshold used for ST segment depression in the Mammohansingh series was lower than that used by Boudoulas. This resulting reduced specificity may account for the patients labelled as having a positive ETT who did not exhibit lactate production.

Greenberg et al demonstrated lactate production in a subgroup of patients with normal coronaries (10 out of 17). This group showed much smaller increases in myocardial blood flow (as measured by thermodilution) compared to those in whom lactate was not seen. They also showed an unchanged coronary vascular resistance in contrast to non-lactate producers who exhibited a 32% decrease in vascular resistance at high rates of atrial pacing (28). Similar findings by Opherk et al demonstrated lactate production in their selected patients with normal coronaries and positive stress test in contrast to their control group, during atrial pacing. A much blunted increase in coronary blood flow (as measured by the argon concentration method) and increase in calculated coronary resistance were also observed in this patient group compared to control subjects (29).

The metabolic evidence for ischaemia in patients with normal coronaries is by no means undisputed. There is at least one report showing no evidence in these patients of abnormal lactate responses despite reduced coronary flow reserve. This was in contrast to those with documented coronary disease in whom abnormal lactate extraction was seen. Only three out of 14 patients with normal coronaries showed a significant drop in coronary sinus pH and coronary sinus oxygen saturation consistent

with ischaemia despite most experiencing anginal pain during pacing and 11 out of 14 developing ischaemic ECG changes (30). Such reports result in ongoing controversy about the ischaemic basis for some patients with 'Syndrome X', but this whole field is likely to remain in dispute until a more sensitive marker for ischaemia is found to act as a 'gold-standard'. The studies looking at lactate as a marker for ischaemia in 'Syndrome X' are summarised in table 2.2 overleaf.

Table 2.2: Summary of the studies looking at myocardial lactate metabolism as a marker for ischaemia in patients with angina and normal coronary arteries

Study	Patient Numbers	Characteristics	Stress	Groups Compared	Results
POSITIVE STUDIES					
Cannon III et al 1988 (31)	115 patients 57% women	Angina and normal CA 10% with +ve ETT 85% with non-diagnostic ETT	Atrial pacing at 150 bpm	87 patients experienced chest pain during pacing (Group A) and 28 did not (Group B)	Group A had significantly lower lactate extraction compared to Group B
Boudoulas et al 1974 (26)	29 patients mean age 41 yrs	Angina and normal CA	Coronary sinus pacing at 160-180 bpm (until chest pain)	9 myocardial lactate producers (Group A) 20 non-producers (Group B)	Significantly more ST-depression in Group A. Every patient with ST depression >2mm was in Group A. Every patient with <1mm ST depression was in Group B.
Mammohansingh et al 1975 (27)	17 patients 94% women	Angina and normal CA and electrically positive stress test	Atrial pacing (mean rate 143 bpm)	14 patients -chest pain 3 patients – no chest pain	4 patients (all in chest pain group) exhibited abnormal lactate metabolism (2 reduced extraction, 2 production)
Greenberg et al 1987 (28)	27 patients 48% women mean age 52 yrs	Angina and normal CA	Atrial pacing at 160 bpm	10 lactate producers (Group A) 17 non-producers (Group B)	Group A had attenuated increases in coronary blood flow during pacing compared to Group B (14% Vs 75%) and no reduction in coronary vascular resistance compared to a fall of 32% in Group B
Egashira et al 1993 (32)	9 patients 38% women mean age 59 yrs	Angina and normal CA with electrically positive stress test	Intracoronary papaverine 10mg	9 patients 6 healthy controls	All 9 patients had net myocardial lactate production associated with chest pain (67%) and ECG changes (67%). No lactate production/chest pain or ECG changes in controls.
Mohri et al 1998 (33)	36 patients 58% women median age 63 yrs	Angina and no more than mild coronary disease (no stenosis >50%)	Incremental dose intracoronary ACh	17 lactate producers (Group A) 19 non-producers (Group B)	All Group A patients had either large artery coronary spasm or 'microvascular spasm' (defined by ECG changes or chest pain without large epicardial spasm)
Cannon III et al 1983 (34)	22 patients 23% women mean age 51 yrs	Angina 73% normal CA 27% with non-obstructive coronary disease	Coronary sinus pacing at 150 bpm	9 patients – chest pain (Group A) 13 patients – no chest pain (Group B)	Group A patients had lower lactate extraction compared to Group B (25.0 Vs 45.6 mmol/L.min). Group A patients also had lesser increases in cardiac vein flow and lesser decrease in coronary resistance

Cannon III et al 1985 (35)	30 patients 55% women mean age 51 yrs	Angina and normal CA	Coronary sinus pacing at 150 bpm after 0.15mg IV ergonovine	22 patients – chest pain (Group A) 8 patients – no chest pain (Group B)	Group A patients had lower lactate extraction compared to Group B (30.5 Vs 69.7 mM.ml/min). Group A patients also had lower cardiac vein flow and higher coronary resistance
Cannon III et al 1985 (36)	50 patients 50% women mean age 49 yrs	82% normal CA 18% <30% narrowing of any CA	Coronary sinus pacing at 150 bpm	24 patients – chest pain (Group A) 26 patients – no chest pain (Group B)	Group A patients had lower lactate extraction compared to Group B (28.3 Vs 51.3 mM.ml/min). Group A patients also had lesser increases in cardiac vein flow and lesser decrease in coronary resistance
Opherk et al 1981 (29)	8 patients 25% women mean age 47.4 yrs	Angina and normal CA 78% with electrically +ve ETT	Atrial pacing at 150 bpm	8 subjects 5 controls (with atypical pain and –ve stress test)	Significant myocardial lactate production in the 8 subjects but not the control group. (Increase in lactate 3.9 Vs 0.8 µmol/100g.min)
NEGATIVE STUDIES					
Botker et al 1997 (37)	18 patients 83% women mean age 54.6 yrs	Angina and normal CA and electrically +ve stress test	Coronary sinus pacing at 150 bpm	18 subjects 10 controls (with atypical pain and –ve stress test)	No differences in lactate metabolism seen between rest and pacing in either group
Camici et al 1991 (38)	12 patients	Angina and normal CA and electrically	Atrial pacing up to 150 bpm	12 subjects 10 healthy controls	No differences in lactate extraction between the 2 groups at any time and no evidence of lactate production in SX group.
Rosano et al 1996 (30)	14 patients 57% women mean age 51 yrs	Angina and normal CA (11/14 with electrically +ve stress test)	Atrial pacing at 160 bpm	14 subjects 9 controls (with coronary disease)	No difference in lactate metabolism for the subjects. In contrast, the controls with coronary disease showed marked reduction in lactate extraction.

ETT – exercise tolerance test
CA – coronary angiogram
ACh – acetyl choline

Coronary Sinus Oxygen Saturation

Crake et al (39) have looked at coronary sinus oxygen saturation as a potential marker of ischaemia. In the normal subject, increasing myocardial demand in response to incremental atrial pacing, leads to a transient fall in coronary sinus oxygen saturation with a return to normal fairly quickly as autoregulation adjusts blood flow. This return to normal is not seen in those with coronary disease, with oxygen saturation remaining low and falling further in response to an increasing heart-rate. This is due to physical obstruction limiting flow in the coronary arteries preventing autoregulation.

A similar fall in coronary sinus oxygen saturation has been observed in some patients with 'Syndrome X' in response to incremental pacing (2 out of 10) but no clear correlation with ECG changes was observed (39).

Markers of Reperfusion Oxidative Stress

One study involving small numbers (9 patients with 'Syndrome X') has shown an association between ST-segment depression and reperfusion markers of ischaemia. Buffon et al measured levels of lipid hydroperoxides and conjugated dienes, as indicators of ischaemia-reperfusion oxidative stress, in the great cardiac vein before and after incremental pacing. All 9 patients showed ST-segment depression and compared with controls had marked increases in the levels of these two markers in the great cardiac vein (40). These markers have been demonstrated in reperfusion during angioplasty in patients with obstructive coronary disease (41), and with their detection in this group with 'Syndrome X' suggests a genuine ischaemic basis for the ECG changes and symptoms in these 9 patients. No other group has looked at these markers to date, and it does seem from their data that these molecules are more sensitive than myocardial lactate for the detection of ischaemia.

Ultrastructure

An even more invasive means for the identification of myocardial ischaemia is endomyocardial biopsy and direct examination of cells for ischaemic damage. Such a technique was employed by Opherk et al with biopsy specimens obtained from 18 patients with 'Syndrome X'. Although specimens looked normal under light microscopy, the majority showed abnormalities under electron microscope examination. This mostly took the form of varying degrees of mitochondrial swelling consistent with subtle ischaemic damage. There were no observed abnormalities of the microvascular vessels (29). However, data from Suzuki et al do suggest some changes around small vessels. This group looked at 21 patients with normal coronary arteries despite ST-segment changes during atrial pacing, and demonstrated sclerosis of arterioles and peri-vascular fibrosis in 95% of subjects. Furthermore, electron microscopy showed proliferated and deformed smooth muscle cells in the media layer of the small vessel walls, suggesting that there may be structural changes in the vasculture of 'Syndrome X' patients (42). Of note, however, neither the study by Orpherk nor the study by Suzuki compared these findings to either normal controls or subjects with coronary disease and known ischaemia.

Summary

From the above data, it is clear that there is certainly a subgroup of patients satisfying the criteria for Syndrome X', who exhibit some metabolic evidence of ischaemia. Even amongst the invasive investigations, no one good sensitive test for detection of physiological ischaemia has emerged and a lot of the controversy that exists in this field is a result of the lack of a good 'gold-standard' for the demonstration of myocardial ischaemia. Although myocardial lactate production perhaps remains the closest test we have to a 'gold standard', even this lacks sensitivity. Furthermore, there is some correlation but no practically useful relationship to ECG changes.

The data available show that we are unable to conclude that all patients with ECG changes will exhibit lactate production as a more direct marker of ischaemia (27). Neither are we able to deduce that those without ECG changes will not produce lactate (26).

Non-Cardiac Pain

It is generally believed that the majority of patients fulfilling criteria for 'Syndrome X' experience non-cardiac pain. There are many other aetiologies which can closely mimic ischaemic-type pain even fulfilling many of Heberden's original characteristics. The task of distinguishing between these patients remains challenging.

Oesophageal Pain

The oesophagus has always been a major culprit implicated by its ability to reproduce typical anginal-type symptoms. Both acid reflux and oesophageal spasm can induce ischaemic-type chest pain. The effect of GTN in relaxing smooth muscle and relieving the pain of oesophageal spasm further confounds the difficulty. When groups of patients with 'Syndrome X' are examined specifically for oesophageal abnormalities, a significant proportion of them will prove positive. Furthermore, simple acid-suppression therapy is often effective in relieving symptoms (43).

To complicate things further, Chauhan et al have described a so-called 'cardio-oesophageal' reflex. They demonstrated that instillation of acid into the oesophagus of 35 'Syndrome X' patients resulted in reduced blood flow in the left anterior descending coronary artery by Doppler measurements, compared to 24 heart transplant patients (with effective cardiac denervation). Although the mechanism is unclear the authors hypothesised that this effect depends upon a neural reflex which is abolished when the heart is denervated (44). Other upper gastro-intestinal pathology can frequently masquerade as anginal-type chest pain and vice-versa. It is as well-recognised phenomenon that some patients presenting with myocardial infarction have symptoms that are compatible with indigestion.

Musculoskeletal Pain

Musculoskeletal chest pain is another important aetiology which must be considered for patients presenting with 'Syndrome X'. A series of 40 such patients (some with minor coronary disease) were examined for rheumatological disorders including fibromyalgia and costochondritis. Such a diagnosis was able to be confirmed clinically in 40% of this group compared to a control group with coronary disease in which only one out of 40 was diagnosed (45).

Psychological Abnormalities

Some take the view that patients with 'Syndrome X' have psychological abnormalities. Wielgosz et al reported that patients with normal coronaries who had ongoing pain, had high hypochondriasis scores and suggested that they has an exaggerated preoccupation with personal health (46) which could lead to an increased tendency to complain about symptoms. This was not examined in relation to a control population and it is conceivable that these patients' anxieties relate to ongoing symptoms, ongoing contact with the medical profession, and invasive investigations. It would be useful to compare hypochondriasis scores with a population with documented coronary disease. Others suggest have suggested that the incidence of pain disorder is higher in this population (47).

Increased Pain Perception

Some have reported alterations in the way this patient group perceive pain suggesting an increased cardiac sensitivity. 29 out of 36 patients (81%) with normal coronaries and chest pain, reported pain during intra-coronary contrast injection and right heart catheter manipulation and pacing, as opposed to only 2 out of 33 with coronary disease. This was despite a higher cutaneous pain threshold in the 'Syndrome X' group and led the authors to hypothesise about neurally-mediated abnormal pain responses to cardiac stimuli in this group (48). Similarly, abnormal cardiac pain perception was hypothesised by another group who were able to reproduce chest pain in 34 out of 36 patients with 'Syndrome X' (94%) with intra-cardiac catheter manipulation compared to much lower figures in patients with mitral valve and

coronary disease (49) . However, these studies were not blinded and are extremely susceptible to reporter bias which may lead to distorted results.

The neural link to pain perception has been examined by functional brain imaging using positron emission tomography (PET), as a measure of regional neuronal activity during chest pain. Patients with 'Syndrome X' tended to have a distinct pattern of brain activity as compared with other groups including those with obstructive coronary disease and silent ischaemia (50). This has led some to conclude that chest pain in this group of patients has a significant 'supra-tentorial' element. In keeping with a neurally-mediated component to pain, Cunningham et al, in a small pilot study, demonstrated positive effects on exercise capacity and quality-of-life indicators in patients with 'Syndrome X' after a three month training course on transcendental meditation (51).

It is therefore evident that while some direct metabolic markers for ischaemia are present in a subgroup of patients with 'Syndrome X', a large proportion also have evidence of non-cardiac organic pathology and others may have psychological components to their pain. Others, still, may contribute to their symptoms by virtue of neurally-mediated abnormalities of increased cardiac sensitivity and or pain perception. This group of patients is a heterogeneous group and discrimination between those with presumed myocardial ischaemia and those without remains very difficult in everyday clinical practice. The studies quoted above, showing potential non-cardiac mechanisms for pain in these patients, all used varying entry requirements and as a result recruited significant numbers of patients with non-cardiac pain. Very few of these trials stuck to rigid criteria including definite demonstration of ischaemia either invasively or non-invasively.

Clinical Modalities for Discrimination between Microvascular Angina and Non-Cardiac Pain

It is neither practical nor ethical to look for markers of ischaemia or signs of coronary vasomotor dysfunction discussed previously in this chapter, when assessing patients with chest pain and normal coronary arteries. It is important to attempt to distinguish between patients with myocardial ischaemia and those with non-cardiac pain. This distinction avoids the incorrect label of 'angina' as this can have significant implications. In fact, many non-cardiac causes are symptomatically treatable and these patients ought not to be exposed unnecessarily to anti-anginals and their potential side-effects, whenever possible.

This requires demonstration of myocardial ischaemia usually by a non-invasive indirect marker. However, this is often a very difficult clinical situation because established indirect, non-invasive markers of ischaemia can prove unhelpful.

Exercise Tolerance Test

This is a well-established test in the investigation of coronary disease but its relative lack of sensitivity means that inevitably some patients with coronary disease fail to exhibit ECG changes during exercise (5). Because of this, it is conceivable that some patients with chest pain and normal coronary arteries will have myocardial ischaemia, despite a negative ETT. This is borne out by some of the pacing/lactate experiments of the 1970s and 1980s, in which it became clear that some of these 'false positive' ETTs occur in patients with more invasive metabolic evidence of ischaemia such as myocardial lactate production (26;27). The data from Boudoulas suggest that, if we assume abnormal lactate metabolism is relatively specific for ischaemia, clear ECG changes on ETT were also specific (all patients with >2mm ST-segment depression produced lactate). However, ST-segment depression lacked sensitivity (especially

with a 2mm threshold), as there were patients who produced lactate (with therefore a presumably ischaemic basis) who did not fulfil ECG criteria. Having a 2mm threshold, although reducing sensitivity does increase specificity for myocardial ischaemia.

Therefore, it seems reasonable to assume that any subject with angiographically normal coronary arteries and clear 2mm of ST-segment depression on exercise ECG is likely to have an ischaemic basis for their symptoms presumably due to microvascular angina. The group with borderline or no ECG changes remains difficult to characterise due to the relative lack of sensitivity of this test. There will be some subjects with microvascular angina and many with non-cardiac pain. This contrasts with the data presented by Mammohansingh in which they used a threshold of ST-segment depression of 0.5mm. Lowering this threshold markedly reduces the specificity of the test with only 4 out of 17 patients in this group exhibiting abnormal lactate responses (27), however, this may be in part due to the lack of sensitivity of lactate production for myocardial ischaemia.

Similar problems were identified by Epstein et al. This group labelled patients with microvascular angina by means of impaired coronary vasodilator reserve (using techniques involving great cardiac vein flow under basal and stimulated conditions) and exercise-induced left ventricular dysfunction. Out of 115 patients categorised in this way, only 10% had ischaemic ST-segment changes on exercise – a sensitivity of less than 9% if one presumes that all 115 of these patients have microvascular angina (52). Cannon et al have also reported figures of over 75% for patients with microvascular angina (characterised by the same abnormal coronary vasodilator responses) having normal exercise ECGs (53). The difficulty in distinguishing those patients with myocardial ischaemia and "false positive" exercise tests due to presumed microvascular disease, from the 'true' false positive exercise tests (patients with non-cardiac pain) was highlighted by this group – if ECG criteria alone are used for the diagnosis of microvascular angina, a large proportion of patients will be missed and many more may be wrongly diagnosed.

In patients with coronary disease, regional wall motion abnormalities as detected by stress Echo are generally seen earlier than ECG changes and therefore tend to be a more sensitive marker for ischaemia. Zouridakis et al looked at dobutamine-stress echocardiography in patients with chest pain and normal coronaries. 33 subjects all with a positive stress test and normal coronary arteries were studied. Although chest pain was reproduced in 14 patients (42%) and ST-segment changes in 15 patients (45%), none of the subjects studied developed significant regional wall motion abnormalities (54).

However, another study using adenosine as pharmacological stress in 9 patients with microvascular angina showed global impairment of diastolic function as manifest by technetium blood-pool imaging and trans-mitral Doppler flow changes. There was no change in systolic function in these patients (55). This diastolic dysfunction was postulated to be a possible manifestation of global subendocardial ischaemia.

LV function as assessed by resting and exercise-gated blood-pool scintigraphy by Cannon et al (in a group thought to have microvascular angina as manifest by normal coronaries and abnormal coronary vasodilator reserve) demonstrated reduced LV ejection fraction in comparison to those without abnormalities in vasodilator reserve and normal subjects (56).

The lack of discrete wall motion abnormality on Echo suggests that either these patients do not develop myocardial ischaemia during the stress Echo or that stress Echo is not sufficiently sensitive to identify ischaemia. This may be true, but obstructive coronary disease is usually focal with segmental variation and this makes it more amenable to producing a regional wall motion abnormality as the affected segments are easily visualised against the normally-moving (non-ischaemic) segments. The functional vasomotor defect in microvascular angina may be a more generalised process and this lack of localisation may make techniques such as stress

Echo less useful. It has been postulated that global diastolic dysfunction and subtle global reductions in LV ejection fraction as assessed by radionuclide blood-pool techniques in the studies above may be markers of ischaemia.

Thallium Scanning

Radionuclide perfusion scintigraphy is a useful modality in demonstrating relative myocardial perfusion defects and their degree of reversibility in the investigation and assessment of coronary disease. Sensitivity of this technique in predicting coronary disease has been reported as high as 91% (57). Its role in identifying patients with myocardial ischaemia amongst those labelled with 'Syndrome X' is less clear. Although perfusion scintigraphy is a relatively sensitive test, it can lack specificity for coronary disease with defects described in patients with left bundle branch block (58), permanent pacemakers (59) and even sarcoidosis (60).

A series of 100 patients with normal coronary arteries and typical angina was looked at by Tweddel et al. They reported varying degrees of abnormal thallium perfusion in 98% of these patients. Out of this group, only 30 had ECG changes on exercise. Although those with ECG changes tended towards bigger defects, no clear correlation between perfusion defects and a positive ETT was observed (61). In a more selected group (with definite ECG changes) thallium scintigraphy identified reversible defects in 18 out of 33 patients (55%) with normal coronaries and chest pain. The presence of systemic hypertension did not influence the rate of thallium defects (54).

Looking at Tweddel's relatively unselected group, it is difficult to believe that 98% of these patients had myocardial ischaemia as a cause of their symptoms and without a control group it is difficult to estimate the prevalence of perfusion defects in the asymptomatic normal population. Nonetheless, it is likely that a significant proportion of this group did have myocardial ischaemia, but it would seem that the relative lack

of specificity of thallium scintigraphy leads to over-representation of abnormal myocardial perfusion in this group of patients.

An Italian group looked a more stringently defined group with 35 patients exhibiting normal coronaries but all with a positive stress test. They included 32 patients with atypical pain and a negative ETT as a control group. Only 3 patients from the control group showed any perfusion defects (4 segments in total) during stress with reversibility in 1 patient and reverse distribution (segments hypo-perfused at rest compared to stress) in another 4 patients. This compares to 27 patients from the patient group in whom stress defects were apparent (40 in total). Over half of the affected segments showed reversibility and there was a much higher prevalence of reverse distribution with 33 segments affected (62).

Earlier studies in the late 1970s and 1980s conflicted as to the prevalence of perfusion defects. Meller et al found only 4 patients (15%) with perfusion defects with only 2 being reversible out of a series of 27 patients selected on the basis of a normal coronary angiogram and chest pain. ECG changes were seen in 10 out of these 27 patients. Looking at the 4 patients with abnormal perfusion imaging, 50% of them had a positive ETT only (63). In a similar population of 41 patients, 11 abnormal thallium perfusion scans (27%) were found by Berger et al. 3 of these 11 with abnormal perfusion imaging had a positive ETT. A significant number of those with perfusion defects had other cardiac abnormalities including bundle branch block and mitral valve prolapse which may contribute to the abnormal imaging (64). Kaul et al found significantly different perfusion in a group of 44 patients with normal and minimally-diseased coronaries and chest pain (9 of whom had positive ETTs) as compared with 45 normal subjects free from chest pain. ECG changes on exercise did not correlate with the presence of abnormal thallium imaging (65).

Thallium scintigraphy appears non-specific and even in an unselected group there will tend to be a very high proportion of 'Syndrome X' subjects with perfusion defects (61). In a more selected group in terms of ECG changes, there does appear to be a difference in the frequency of perfusion defects as compared to a 'control' group with

atypical pain. The difficulty in this area is that perfusion defects have never been correlated with more definite invasive measures of ischaemia such as abnormal myocardial lactate metabolism. Due to this, studies have compared perfusion defects on thallium scintigraphy to ECG changes, which are known to be lacking in sensitivity and specificity. Very little in the way of definite conclusions as to the usefulness of thallium scanning in the 'Syndrome X' population, can therefore be drawn from the available data. It does appear that a positive result will not aid discrimination between cardiac and non-cardiac pain and there are no strong data to suggest that a negative result seems to be more predictive of a non-cardiac aetiology.

Magnetic Resonance Imaging/Nuclear SPECT

Magnetic resonance imaging (MRI) is a high resolution technique that potentially may be more sensitive to ischaemic changes within the myocardium. It has been shown using MRI quantitative perfusion index, that patients with 'Syndrome X' fail to augment myocardial blood flow in the sub-endocardial region but augment normally epicardially in response to intravenous adenosine. Normal augmentation was seen both in the endocardium and epicardium in normal control subjects. These data do suggest that these patients fail to vasodilate normally in the subendocardium (66).

A relatively new technique uses phosphorous-31 nuclear magnetic resonance (^{31}P-NMR) spectroscopy to identify metabolic evidence of ischaemia non-invasively. Phosphocreatine is required within the myocardium for normal function, and it has been demonstrated in animals, that a loss of phosphocreatine (PC) and a decrease in the phosphocreatine : adenosine triphosphate (ATP) ratio occurs during myocardial ischaemia (67). ^{31}P-NMR spectroscopy has been employed in patients with coronary disease successfully to demonstrate transient ischaemia during hand-grip exercise (68).

Recently its use in evaluating ischaemia in women with chest pain and normal coronaries has been studied. In a group of 35 women, it was found that 20% showed

non-invasive evidence of ischaemia by this method, when compared with age-matched controls (69). This group were not selected for by ECG changes, and it is therefore likely that the relatively low proportion of women showing evidence of ischaemia reflects the likelihood of non-cardiac pain in the majority of these patients. Further work correlating ECG changes with this method would be useful.

Aetiology and Pathogenesis of Microvascular Angina

Although still a contentious issue, most now believe that myocardial ischaemia is at least a contributing factor for a subgroup of patients with normal coronaries and typical anginal pain. How they are further characterised in terms of a positive ETT, reversible scintigraphy defect, direct or indirect metabolic markers, stress LV dysfunction or even abnormal coronary vasodilator reserve, remains even more controversial because no one test has reliable levels of sensitivity or specificity and therefore a 'gold-standard' is lacking. The approach adopted by most clinicians is one of excluding other obvious causes of chest pain and employing other available investigations such as the ETT and perfusion scintigraphy in combination perhaps more than once.

Vasomotor Dysfunction

Accepting an ischaemic basis after the demonstration of normal epicardial arteries in the absence of inducible spasm, would seem to implicate the coronary microcirculation by default as responsible for limitations on coronary blood flow. This is the view taken by most investigators (31) and some believe that it is specifically the arterioles at the level of approximately 200 micron diameter that are dysfunctional. In fact some groups have used inclusion criteria based on abnormal coronary flow reserve when conducting studies to further characterise these patients with presumed microvascular angina (53).

The hypothesis adopted by most investigators in this field is that angina in these patients is due to vasomotor abnormalities which lead to a reduced vasodilator capacity. Therefore, a relative shortage of blood flow during times of increased metabolic demand leads to ischaemia. Such abnormal vascular responses have been documented in the coronary vascular bed by several groups.

Impaired Coronary Vasodilator Response

Abnormalities of myocardial lactate metabolism as a marker for ischaemia have been documented by several groups in conjunction with an impaired coronary blood flow response to vasodilator stimuli, in patients with 'Syndrome X'. These studies include those by Greenberg et al (using thermodilution with incremental pacing) (28), Opherk et al (measured by the argon method in response to dipyridamole infusion) (29) and Cannon et al (by great cardiac vein catheterisation and atrial pacing) (36).

This group of studies tends to suggest that this impaired vasodilator response is metabolically significant in the production of ischaemia. Moreover, there some evidence to suggest that this impaired coronary vasodilator response is associated with sub-angiographic coronary disease. Wiedermann et al undertook a study of coronary vasodilation as assessed by ultrasound diameter measurements of coronary arteries during exercise. All patients studied fulfilled the diagnostic criteria for cardiac 'Syndrome X' but only those patients with sub-angiographic atheroma and intimal thickening, as assessed by intra-vascular ultrasound, demonstrated coronary vasoconstrictor responses to exercise. Those with normal coronary intra-vascular ultrasound studies showed normal vasodilator responses during exercise. (14). This tends to suggest that the abnormal vasomotor responses are associated with evidence of sub-angiographic coronary disease.

Several groups have looked at coronary vascular flow reserve in subjects with 'Syndrome X' and some important studies are summarised below in table 2.3.

Table 2.3: summary of studies looking at coronary vascular dysfunction in 'Syndrome X'

Study	Patient Group	'Control Group'	Modality used to Augment Blood Flow	Techniques used	Results
Greenberg et al 1987 (28)	10 pts with chest pain and normal CA - with definite ischaemia (produced lactate)	17 pts with chest pain and normal CA - without definite ischaemia (did not produce lactate)	Atrial pacing at 160 bpm	CBF using thermodilution and CVR	10 pts with definite ischaemia had attenuated increases in CBF (14% Vs 75%) and no reduction in CVR (Vs 32% reduction) with pacing compared to those without definite ischaemia
Egashira et al 1996 (70)	8 pts with SX	8 controls with atypical chest pain and normal CA	i.c. ACh (also given i.c. papaverine and isosorbide dinitrate (ISDN)	CA diameter using angiography and CBF using i.c. Doppler	no significant increase in CBF after i.c. ACh Vs significant increase in control group. Similar increase in CBF seen with i.c. ISDN and papaverine in pt and control groups.
Wiedermann et al 1995 (14)	18 pts with SX and some degree of sub-angiographic disease on IVUS (Group A)	12 pts with SX but without sub-angiographic disease on IVUS (Group B)	Supine arm exercise during angiography calculation of coronary lumen area	Calculation of coronary lumen area	Group B had coronary vasodilation on exercise Vs Group A in whom vasoconstriction of approx 17.5% was seen with exercise
Motz et al 1991 (71)	23 pts with SX	no comparison group	i.c. ACh and iv dipyridamole	CBF determined by gas-chromatographic argon method	17 pts had no significant increase in CBF (>100% resting value) or a vasocontrictive response. Only 6 pts had significant increase in CBF with ACh. 6 pts did not show significant increase in CBF with ACh or dipyridamole.
Egashira et al 1993 (32)	9 pts with SX	10 controls with atypical chest pain and. normal CA and – ve ETT	i.c. ACh (also given i.c. papaverine and isosorbide dinitrate (ISDN)	CA diameter on angiography and CBF using i.c. Doppler	Reduced ACh-mediated increase in CBF in SX group compared to controls (103% Vs 345% at top dose i.c. ACh). No difference in papaverine/ISDN-mediated vasodilation

Chauhan et al 1997 (72)	35 pts with SX	17 controls with atypical chest pain and. normal CA and – ve ETT	i.c. ACh (also given i.c. papaverine / glyceryl trinitrate)	CA diameter on angiography and CBF using i.c.Doppler	Reduced ACh-mediated increase in CBF in SX group compared to controls (124% Vs 361% at top dose i.c. ACh). Reduced vasodilation to papaverine also (185% Vs 411%).
Sanderson et al (73)	12 pts with SX	Compared against 'normal' response of vasodilation 12% +/- 1%.	Cold pressor test	CA diameter on angiography. CBF and CVR also calculated	Reduced coronary vasodilation in all 12 pts (7/12 had vasoconstriction or no change at all) – compared to normal response. Also a fall in CBF and an increase in CVR
Sutsch et al 1992 (74)	12 patients with 'microvascular angina'	6 controls	0.5mg/kg iv dipyridamole	CFR by coronary sinus thermodilution	Increased improvement in coronary flow in control group (290%) compared to patient group (130%)

Pts – patients
ACh – acetylcholine
i.c. – intracoronary
CVR – coronary vascular resistance
CBF – coronary blood flow
SX – syndrome X (angina, normal coronary arteries and positive exercise tolerance test)
ETT – exercise tolerance test
CA – coronary artery
CFR – coronary flow reserve
IVUS – intravascular ultrasound

Abnormalities to pharmacological vasodilators have been reported in the coronary circulation in some patients with 'Syndrome X'. These are summarised in Table 3 also. All studies demonstrate abnormalities of endothelium-dependent vasodilation (mostly in response to intracoronary ACh) (32;70-72). However, results are mixed when it comes to characterising the endothelium-independent response. At least 2 groups have shown no difference in the vasodilatory response to intracoronary nitrate in patients with 'Syndrome X' compared to controls (32;70) but others have shown impairment in this response with endothelium-independent vasodilators, suggesting that vascular dysfunction may extend to the smooth muscle and not just involve the endothelium (71;72;74).

A Generalised Systemic Vasodepressor Response?

There is now much data supporting vascular dysfunction in patients with 'Syndrome X' outwith the coronary vascular bed. Some of these data are summarised in table 4 below. Patients with chest pain and normal coronary arteries, already characterised as having attenuated coronary vasodilation in response to rapid atrial pacing and dipyridamole infusion, also exhibited reduced peripheral vasodilator responses to forearm ischaemia, as measured by plethysmography, compared to normal controls. Furthermore, there was a significant correlation between the attenuation in coronary and peripheral vasodilation (75).

Several groups have documented attenuated brachial artery 'endothelium-dependant' vasodilator responses (reactive hyperaemia post ischaemia) in patients with 'Syndrome X' as compared with normal subjects. These responses were measured by high-resolution ultrasound and no differences in response to sublingual nitrate was noted between the groups (76-78).

One group looking at flow-mediated dilatation post forearm ischaemia using laser Doppler fluximetry did not find any differences between subjects with 'Syndrome X' and controls despite significant differences in their coronary vasodilator reserve, as assessed by coronary sinus thermodilution techniques (74). They did note, however, that resting peripheral flux (degree of vasodilation) was lower in the subjects with microvascular angina, although the hyperaemic response did not differ. This difference in resting vascular tone itself may have some significance.

The endothelium-independent response in this group of studies was looked at using sublingual nitrate in most cases. No significant difference in the nitrate response was seen in these studies leading the authors to conclude that there was no difference in the endothelium-independent response (76-78), in contrast to some of the coronary studies above.

Table 4: Summary of trials looking at peripheral vascular function in patients with 'Syndrome X'.

Study	Patient group	Control group	Technique used	Results
Bellamy et al 1998 (77)	7 pts with SX 57% women mean age 58 yrs	10 normal age-sex matched controls	FMD of the brachial artery in response to hand ischaemia (reactive hyperaemia) and calculation of blood flow using Doppler	Negative FMD in SX group compared to controls (-0.78% Vs +3.46%) $p<0.05$. No difference in response to sublingual GTN. No difference in blood flow changes.
Botker et al 1996 (76)	21 pts with SX 76% women mean age 56 yrs	20 normal age/sex matched controls	FMD of brachial artery in response to forearm ischaemia (reactive hyperaemia) and calculation of blood flow using Doppler .	No difference in FMD but significantly higher increase in blood flow in controls compared with SX group (452% Vs 342%) – $p<0.05$. No difference in dilatation in response to sublingual GTN.
Lekakis et al 1998 (78)	11 women with SX (Group A) mean age 60.2 yrs	2 age-matched control groups: 11 women with 3-vessel coronary disease (group B) 11 healthy women (group C)	FMD of brachial artery in response to forearm ischaemia (reactive hyperaemia)	Significantly increased FMD in healthy controls (7.9%) compared to SX group (1.9%) – $p<0.05$. Attenuation in FMD in SX group similar to that seen in group with coronary disease (3.3%) – p=ns. No significant difference in dilatation after sublingual GTN in all 3 groups.
Kidawa et al 2003 (79)	52 pts with SX 65% women mean age 45 yrs	40 healthy controls sex-matched but on average 4 years younger	FMD of brachial artery in response to forearm ischaemia (reactive hyperaemia) and measurement of Doppler pulsed wave velocity between carotid and femoral arteries (measure of arterial stiffness)	Smaller increase in FMD of SX group compared to controls (6.6 Vs 11.1%) – $p<0.001$. Similar vasodilation seen after sublingual GTN. Increased pulsed wave velocity values in SX group (9.3 Vs 8.2 m/s) – $p<0.001$
Sax et al 1987 (75)	16 pts with SX 56% women mean age 51 yrs	16 normal age/sex matched controls	Forearm plethysmography in response to forearm ischaemia (reactive hyperaemia)	Reduced peak forearm flow in SX group compared to controls (39.9 Vs 31.7 ml/min.dl) – 21% reduction – $p<0.05$
Sutsch et al 1992 (74)	12 pts with 'microvascular angina'	6 control subjects	laser Doppler fluximetry in response to forearm ischaemia	Similar hyperaemic response in both groups but resting cutaneous Doppler flux greater in patient group

SX – 'Syndrome X' – angina with normal coronary arteries and a positive stress test (>1mm ST-segment depression)
FMD – flow-mediated dilatation
GTN – glyceryl trinitrate

If such a correlation exists, then assessment of patients with 'Syndrome X' would potentially be revolutionalised. Although measuring coronary flow reserve invasively has been used extensively as a research tool, it is unlikely to ever enter routine clinical practice. If peripheral vascular dysfunction could be used as a surrogate marker for vascular dysfunction in the coronary bed, it would perhaps be possible to categorise patients with cardiac 'Syndrome X' as having microvascular angina or non-cardiac pain on the basis of their peripheral vasomotor response.

However, whether or not there is any correlation between the vasomotor responses in the peripheral and coronary vascular beds remains inconclusive. Bottcher et al used high-resolution ultrasound to measure brachial artery vasodilation and brachial artery blood flow, post transient forearm ischaemia (reactive hyperaemia). They also looked at myocardial blood flow increase in response to dipyridamole infusion using positron emission tomography (PET). They found no correlations between the peripheral and coronary response in subjects with coronary disease, cardiac 'Syndrome X' nor healthy controls (80). A similar negative result was reported by Sutsch et al, albeit in small numbers, calculating coronary flow reserve by thermodilution (in response to dipyridamole) and comparing to cutaneous laser Doppler fluximetry (74).

However, earlier data from Anderson et al did show some association between the behaviour of coronary and brachial arteries in patients undergoing coronary angiography. This group looked at the coronary vasomotor response to intra-coronary acetylcholine (ACh) and found that those subjects whom exhibited a coronary vasoconstrictor response, had attenuated brachial artery flow-mediated vasodilatation, as assessed by high resolution brachial artery ultrasound (4.8% Vs 10.5%) , compared to those who had a coronary vasodilation response to ACh (81). More support for a correlation between coronary and peripheral vascular responses comes from data by Sax et al. They calculated minimum peripheral resistance after forearm ischaemia using plethysmography as well as minimum coronary resistance, calculated by coronary blood flow determined by thermodilution in response to intravenous

dipyridamole. Their results showed a robust correlation between peripheral minimum resistance and coronary minimum resistance (r=0.74) for 13 subjects in whom data were collected (75).

Much of the controversy in this area may relate to the type of vasculture studied. Anderson et al compared brachial artery function to coronary artery function and as these 2 arteries are in the same order of diameter, a significant correlation was found. However, Bottcher's group was looking at coronary flow in response to dipyridamole by PET, and this is much more likely to be influenced by smaller vessels at the arteriole level. It may, therefore, be no surprise that this was not correlated to brachial artery function in any group of patient studied. When using a technique such as PET or coronary thermodilution to make measurements of coronary flow, a peripheral measurement such as plethysmography or laser Doppler may be better suited to make direct comparisons, as was used in the Sax data (75).

Although the weight of evidence does appear to support a generalised systemic vasomotor disturbance in patients with presumed microvascular angina, there still remains some conflicting evidence. Whether such a systemic disorder is present in 'Syndrome X' has important implications in future testing as discussed earlier – if these vasomotor disturbances are able to characterise patients with microvascular angina, it may be possible to use them in order to aid diagnosis in patients with 'Syndrome X' and help discriminate between those with non-cardiac pain. Invasive tests of coronary vascular function are inappropriate as a clinical tool but systemic cutaneous measures of vascular function are much less invasive and may be adapted for clinical use in the future.

Insulin Resistance

Several groups have demonstrated insulin resistance in patients with 'Syndrome X' relative to control subjects. The hyperinsulinaemic euglycaemic clamp is the gold standard technique for measuring insulin resistance and has been employed by several investigators. Several groups have demonstrated reduced whole body glucose uptake in patients with 'Syndrome X' compared to controls suggesting relative insulin resistance (82-84).

Other groups have demonstrated insulin resistance using surrogate markers including the insulin response to an intravenous glucose tolerance test in postmenopausal women (85), the minimal model assessment of insulin resistance in non-obese men and a mixed sex group (86;87), and post-glucose load insulin levels in a mixed sex group (88-90). In addition, several groups have not only demonstrated higher indices of insulin resistance but also significant dyslipidaemia in patients with 'Syndrome X' as compared to healthy control subjects (85-87;90).

However, there are reports in the literature of no association between 'Syndrome X' and insulin resistance, one using the clamp technique (91) and the other making use of the minimal model of assessment of insulin resistance (92). The balance of evidence, however, does favour this link to insulin resistance and the negative findings of the 2 studies may reflect the heterogeneity within this group of patients or patient selection. These findings are summarised in table 5 below.

The implications of insulin resistance within this group are important because of the potential link to endothelial dysfunction. A close correlation between insulin sensitivity and endothelial nitric oxide (NO) synthesis is seen in healthy volunteers (93). A similar correlation is seen between insulin sensitivity and vasoconstrictor responses to N-monomethyl-L-arginine (an NO inhibitor), in a mixed group of men

Table 5: Summary of trials looking at indices of insulin resistance in patients with 'Syndrome X'.

Study	Patient Group	Control Group	Assessment of Insulin Resistance	Results / Comments
POSITIVE TRIALS				
Botker et al 1993 (82)	11 patients with >1mm ST-segment depression or LBBB development on ETT and normal CA 64% women mean age 55 yrs	9 controls with non-cardiac pain 75% women mean age 54 yrs	Hyperinsulinaemic , euglycaemic clamp (0.8mU/kg per min)	GDR 2.9 mg/kg per min in patient group Vs 4.9 mg/kg per min in control group (41% reduction in SX group ($p<0.01$)
Vestergaard et al 1995 (84)	9 patients with >1mm ST-segment depression on ETT and normal CA 78% women mean age 48 yrs	7 controls with chest pain but normal ETT and normal CA 86% women mean age 48 yrs	Hyperinsulinaemic , euglycaemic clamp (2mU/kg per min	Significantly higher fasting insulin in SX group compared to controls (43 Vs 22 pmol/L. ($p<0.02$) GDR 13.4 in SX group compared to 18.2 mg/kg per min in control group – 26% reduction in SX group ($p<0.02$)
Botker et al 1997 (83)	8 patients with chest pain, >1mm ST-segment depression on ETT and normal CA 75% women mean age 54 yrs	8 subjects with atypical chest pain, normal ETT and normal CA	Hyperinsulinaemic , euglycaemic clamp (0.8mU/kg per min)	Whole body glucose uptake lower in SX group (15.6) than in controls (23.1 µmol/kg/min) – 32% reduction ($p<0.05$)
Godsland et al 1995 (85)	20 postmenopausal women with chest pain, >1mm ST-segment depression on ETT and normal CA mean age 58 yrs	20 healthy postmenopausal women attending menopause clinic mean age 57 yrs	Minimal model by IV glucose tolerance test	Incremental insulin area 27.6 in SX group compared to 19.8 pmol/L per min in control group (39% increase) Si 1.89 in SX group Vs 3.09 ml/µU per min in control group (39% reduction) – $p<0.05$
Swan et al 1994 (86)	14 non-obese men with chest pain, >1mm ST-segment depression on ETT and normal CA mean age 46.6 yrs	38 healthy male volunteers mean age 47.3 yrs	Minimal model by IV glucose tolerance test	Fasting insulin 30% higher in men with SX ($p<0.05$) Si 31% lower in men with SX ($p<0.05$)
Botker et al 1997 (87)	20 patients with chest pain, >1mm ST-segment depression on ETT and normal CA 75% women mean age 56 yrs	2 age/gender matched control groups – 20 healthy volunteers and 15 patients with vasospastic angina	Minimal model by IV glucose tolerance test	Si lower in SX group (0.86 – 41% lower) and vasospastic angina group (0.96 – 35% lower) than in control group (1.47 min^{-1}/per pmol/L x 10^{-4}) $p<0.05$

Chauhan et al 1994 (88)	17 patients with positive ETT and normal CA 53% women mean age 49.3 yrs	2 age/gender matched control groups – 17 healthy volunteers and 17 patients with coronary disease	Insulin response to oral glucose tolerance test	AUC insulin significantly higher in SX patients (6.89 – 58%) and CAD patients (9.63 – 121%) compared to controls (4.35 min.µmol/ml x 10^3) $p<0.05$
Alexopoulos et al 1994 (89)	21 patients with SX (angina, +ve stress test and normal CA) 62% women mean age 53.9 yrs	21 healthy volunteers 62% women mean age 51.6 yrs	Insulin response to oral glucose tolerance test	Median insulin values higher in SX group at 60 mins (59% higher – $p<0.01$) and at 120 mins (112% higher – $p< 0.005$)
Dean et al 1991 (90)	11 patients with chest pain, >1mm ST-segment depression on ETT and normal CA 36% women mean age 50.6 yrs	11 healthy volunteers (age and gender-matched)	Insulin response to oral glucose tolerance test	AUC insulin significantly higher in SX patients (10.44) than controls (6.71 min.mU/L x 10^3) – 56% increase ($p<0.01$)
Fuh et al 1993 (94)	20 patients with angina, +ve ETT and normal CA 60% women mean age 56 yrs	20 healthy volunteers 60% women mean age 55 yrs	Insulin response to oral glucose tolerance test	Patients with SX relatively hyperinsulinaemic and hyperglycaemic during oral glucose tolerance test compared to controls ($p<0.001$)
NEGATIVE TRIALS				
Quionones et al 1996 (91)	10 patients with chest pain, >1mm ST-segment depression on ETT and normal CA 90% women mean age 54 yrs	13 matched healthy controls 77% women mean age 53 yrs	Hyperinsulinaemic , euglycaemic clamp (1.0 mU/kg per min)	Almost identical GDR in patients and controls (25.9 Vs 27.2 µmol/kg per min
Cavallo Perin et al 2000 (92)	10 patients with chest pain, >2mm ST-segment depression on ETT and normal CA 50% women mean age 56 yrs	10 healthy control subjects 60% women mean age 46 yrs	Minimal model by IV glucose tolerance test	No differences in Si between the SX and control groups (5.76 Vs 7.54 min.µmol/ml x 10^{-4}), nor any difference for AUC (insulin)

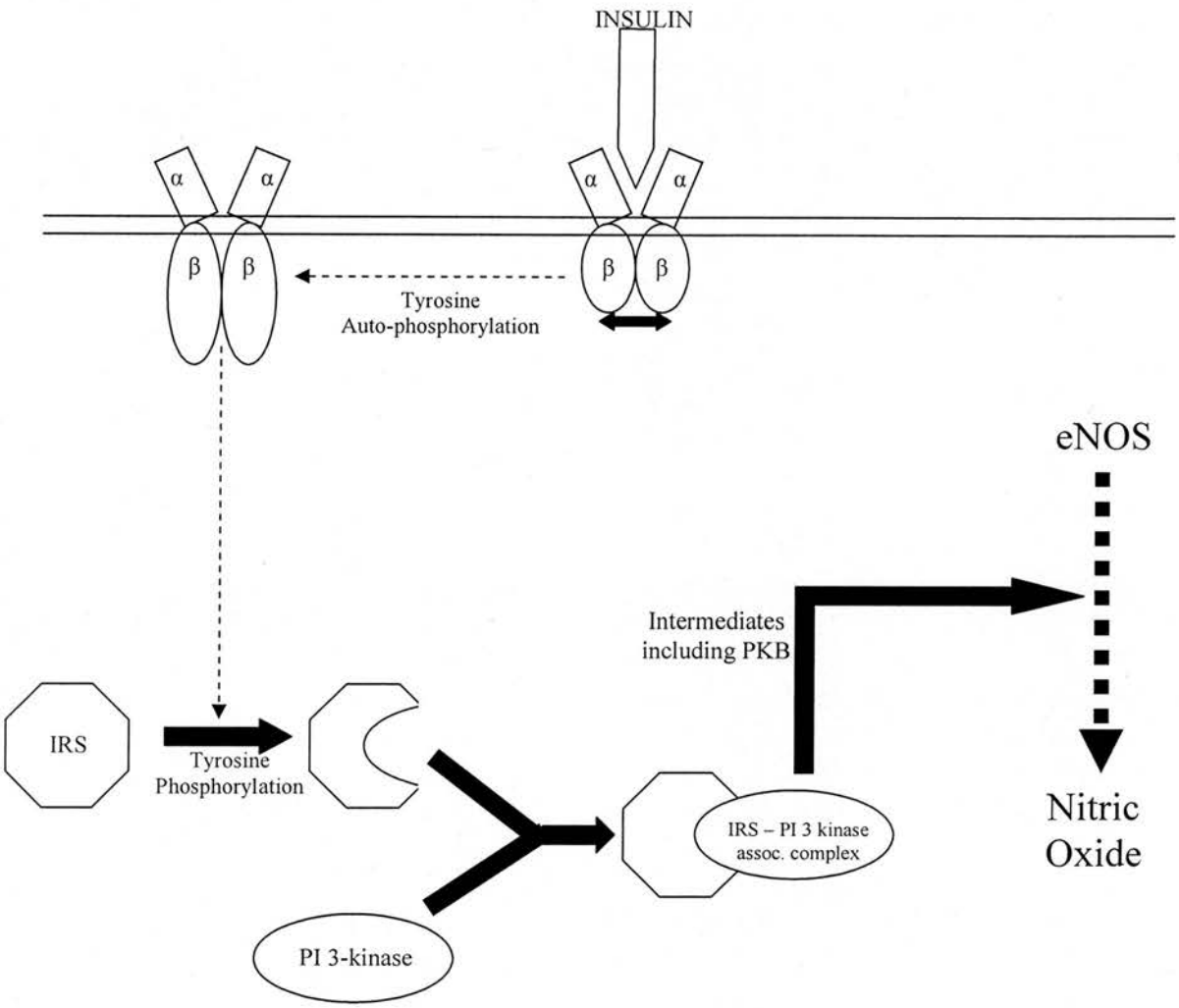
CA – Coronary arteries
SX – Syndrome X
AUC – Area under the curve
Si – insulin sensitivity
ETT – exercise tolerance test
GDR – glucose disposal rate

Including patients with diabetes, hypertension and healthy volunteers (95). Furthermore, the vasodilating effect of insulin is amplified in the presence of metabolically active glucose in healthy subjects (96). This observation, together with findings that insulin augments blood flow in lean subjects points towards a key role for insulin and glucose metabolism in maintaining vasodilator tone in vessels via NO. This is supported by the observation that exogenous insulin therapy appears to improve endothelial function in patients with type 2 diabetes (97).

The post-receptor signalling mediators involved in the production of NO in response to insulin in many ways parallels those which regulate insulin-mediated glucose transport via GLUT4 translocation. It appears that activation of phosphatidylinositol 3-kinase (PI3-kinase) is crucial in this signalling pathway. Cells expressing an inhibitory mutant of PI3-kinase have a much attenuated NO response to insulin. Downstream mediators of this effect may include protein kinase B (PKB) also known as Akt (98), possibly by phosphorylating endothelial nitric oxide synthase (e-nos) (99) – see figure 2. However, other pathways may also be involved and remain to be characterised.

It is therefore possible that insulin resistance may contribute to the endothelial dysfunction observed in patients with 'Syndrome X'. Whether it is important in the pathogenesis of microvascular angina remains to be elucidated. No trials have looked at insulin-sensitising modalities in relation to 'Syndrome X, and such studies looking at endothelial function, subjective assessment to symptoms and objective measures of myocardial ischaemia would be helpful and would potentially advance therapeutic possibilities.

Figure 2
Schematic outlining insulin receptor and NO production coupling in endothelium



IRS: insulin receptor substrate
PI 3-kinase: phosphatidylinositol 3-kinase
PKB: protein kinase B
eNOS: endothelial nitric oxide synthase

Serum Markers of Endothelial Dysfunction

Endothelin-1

There is much data in the literature pertaining to abnormalities of Endothelin-1 (ET-1) in patients with 'Syndrome X'. ET-1 is a potent vasoconstrictor and works in tandem with nitric oxide in maintaining normal vascular tone (100). Several groups have demonstrated significantly higher circulating levels of this peptide in this patient group compared to healthy individuals under resting conditions both in venous and arterialised blood. Furthermore, there are data showing that the augmentation in ET-1 release to stresses, is higher in the 'Syndrome X' group compared with controls. These 'stresses' include pacing-induced tachycardia and a glucose load as part of an oral glucose tolerance test. Much of these data are summarised below in table 6. There are data showing that ET-1 may be elevated as a response to ischaemia (101), but it would be difficult to explain the higher basal levels of ET-1 seen in patients with 'Syndrome X' on this basis.

Data from Cox et al suggest that in fact endothelin levels may correlate with the degree of coronary vascular dysfunction. They showed a robust association between baseline arterial endothelin-1 levels and the fall in coronary vascular resistance (r^2 = 0.44) during fast atrial pacing in 19 patients with angina and angiographically normal coronary arteries. This relationship was indicative of a smaller fall in coronary vascular resistance with greater levels of ET-1 at baseline (102). These data suggest that as well as impaired vasodilation in part due to reduced NO production potentially as a consequence of insulin resistance, a component of active vasoconstriction (as a consequence of increased ET-1) may play a role in the abnormal vascular responses seen in patients with 'Syndrome X'.

It is likely that the increased levels of ET-1 are due to production within the arterial tree, rather than a reduction in the clearance of ET-1 from the venous circulation. This is supported by data showing a higher level of ET-1 within the arterial system compared to the venous system in patients with 'Syndrome X', which is the opposite of what is seen in healthy controls (103).

There is some preliminary data suggesting that hyperinsulinaemia in conjunction with hypertriglyceridaemia may have a causal role in the elevation of endothelin-1 (103). Insulin resistance is a well-recognised feature of cardiac 'Syndrome X' as discussed in table 5. However, it is also known that patients with cardiac 'Syndrome X' do share other features of the Metabolic Syndrome and there are certainly reports of hypertriglyceridaemia in the cardiac 'Syndrome X' cohorts that have been studied (87;104). These metabolic perturbances may have a causal effect in the generation of abnormally high circulating endothelin-1 levels in cardiac 'Syndrome X'. (85), and therefore be directly related to vasomotor dysfunction in this way.(86)

One group did not find any significant difference in the venous levels of ET-1 between patients with 'Syndrome X' and healthy controls. They did however show reduced vasoconstriction peripherally in response to administered ET-1 in patients with 'Syndrome X'. They suggested that in these patients, over-activity of the endothelin system had resulted in downregulation of the endothelin receptor in the peripheral vasculture (105).

All of these data point to an important role for endothelin-1 in modulating the vascular responsiveness seen in patients with 'Syndrome X'.

Other Serum Markers

As well as abnormalities of Endothelin-1 there are data demonstrating increases in other serum endothelial cell markers including Vascular Cell Adhesion Molecule (V-CAM), Intercellular Adhesion Molecule (I-CAM), von Willebrand Factor (vWF) and Plasminogen Activator Inhibitor-1 (PAI-1) in patients with 'Syndrome X', compared to healthy controls (106;107). These findings are not unanimous among the studies in this area with data from some groups showing no difference in some of these markers (104). This suggests that as well as endothelial cell dysfunction manifesting as vasomotor abnormalities in 'Syndrome X', there may be a more generalised problem affecting endothelial production of mediators.

Table 6: Summary of trials looking at endothelin levels in patients with 'Syndrome X'.

Study	Patient group	Control group	Technique used	Results
Lanza et al 1999 (108)	13 pts with SX 77% women mean age 52 yrs	8 age/sex-matched controls undergoing electrophysiological testing	Atrial pacing at up to 160bpm with samples collected from coronary sinus and femoral artery	ET-1 levels higher in SX pts (arterial and coronary sinus). Significant increase in ET-1 in coronary sinus of SX pts only, after pacing (2.01 Vs 2.22 pg/ml)
Kaski et al 1995 (109)	40 pts with angina and normal CA (93% with +ve ETT) 75% women mean age 56 yrs	21 age/sex-matched healthy controls	venous plasma ET-1 levels under resting conditions	ET-1 levels higher in SX group compared to controls (3.84 Vs 2.88 pg/ml)
Kaski et al 1998 (110)	24 pts with SX and 16 pts with angina, normal coronary arteries and negative ETT	21 age/sex-matched healthy controls	Measurement of plasma ET-1 under resting conditions	Plasma ET-1 higher in SX group compared to controls (3.7 Vs 2.96 pg/ml) – p=0.02, but no difference between controls and pts with negative ETT
Piatti et al 1999 (111)	13 pts with SX 62% women mean age 52 yrs	2 age/sex-matched control groups: 13 healthy controls 9 pts with metabolic syndrome (MS)	Measurement of basal 'arterialised' ET-1 and nitrite/nitrate levels	Higher ET-1 levels in SX group compared to controls (8.19 Vs 3.67 pg/ml0 – p<0.01. Similar finding in MS group. However, nitrite/nitrate levels higher in MS group compared to SX/controls
Desideri et al 2000 (104)	24 pts with SX 88% women mean age 54 yrs	14 age/sex-matched controls	Measurement of plasma ET-1 levels under resting conditions and after 75mg glucose load	Plasma ET-1 similar at baseline. Significantly higher ET-1 at 90mins post glucose-load in SX patients compared to controls (p<0.010)
Newby et al 1998 (105)	10 pts with X 73% women mean age 56 yrs	11 age/sex matched healthy controls	venous plasma ET-1 levels under resting conditions	no difference in ET-1 levels between SX and controls (4.8pg/ml Vs 4.0pg/ml)

SX – Syndrome X (angina, with normal coronary arteries and at least 1mm ST depression on treadmill test)
ET-1 – endothelin-1
Pts - patients

Oestrogens

From the epidemiological data, 'Syndrome X' appears to be a female dominated condition when ECG criteria are strictly applied. This has led some to suggest a hormone-related aetiology, specifically that 'Syndrome X' is an oestrogen-deficient state. This view is supported by the observation that the majority of women with 'Syndrome X' experienced symptom onset around the time of menopause and that the incidence of hysterectomy in this patient group has been observed to be four times that of the general population (112). Furthermore, there have been reports of improvement in symptoms and ECG changes in women treated with hormone replacement therapy (HRT) (113;114).

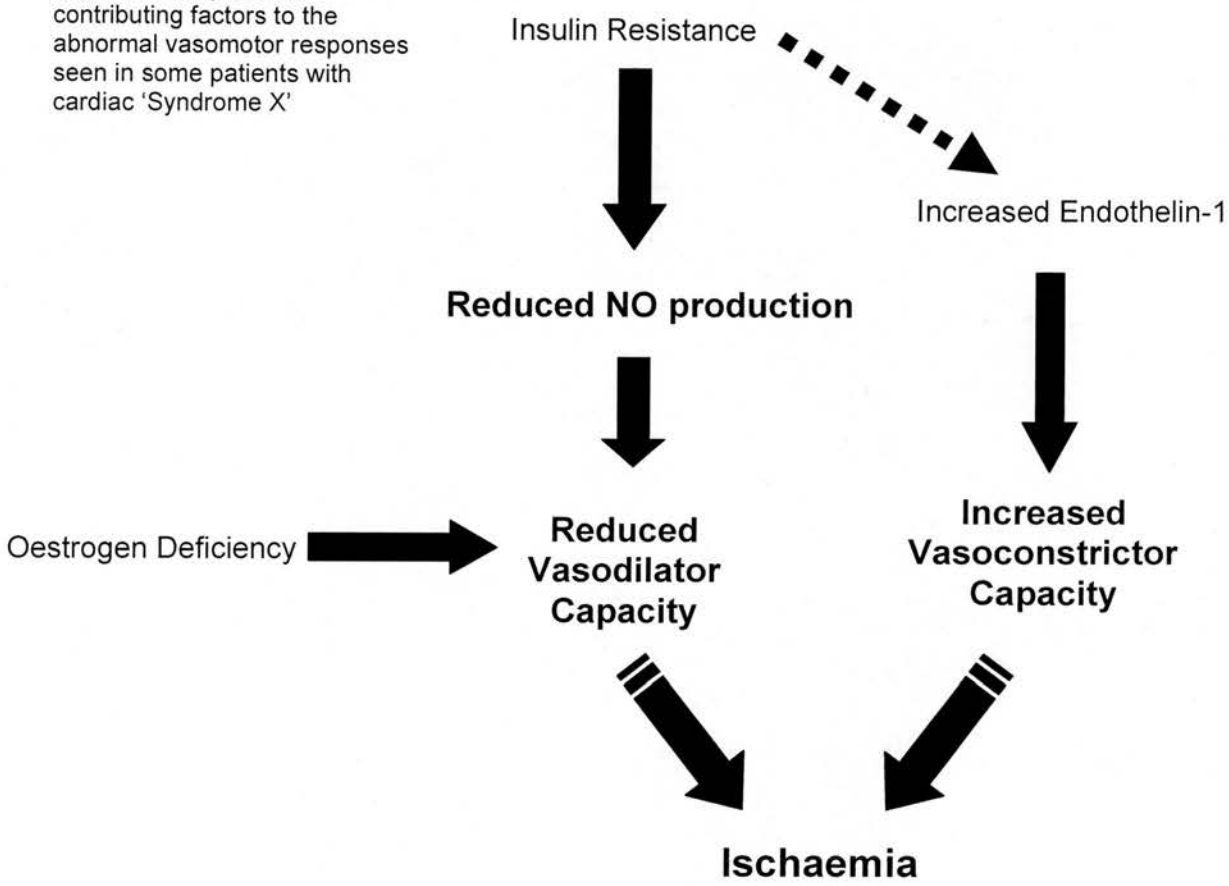
The vasoactive properties of oestrogens are well-documented. It has been shown, using plethysmography, that sublingual oestradiol augments peripheral blood flow in postmenopausal women (115). Furthermore, intra-arterial oestrogen also potentiates the vasodilator effects of intra-arterial ACh and nitrate acutely, with more pronounced effects on those with vascular risk factors (116). The effects on the coronary vascular bed are also favourable with intra-coronary oestradiol reversing the coronary vasoconstrictor response and potentiating the increase in coronary blood flow after ACh (ultrasound measurements) in postmenopausal women with coronary atheroma (117;118).

With such documented acute improvements in vasodilatation with oestrogens, it can be seen why oestrogen deficiency has been implicated as a contributor to vascular dysfunction which may lead to ischaemia as a result of reduced vasodilator capacity. In fact, the administration of HRT transdermally both acutely and in the short-term (6 weeks) has been shown to improve peripheral vascular function by means of high resolution brachial artery ultrasound (119). However, this improvement in endothelial dysfunction is probably common to all postmenopausal women after HRT, not just those with 'Syndrome X'. Whether it affects pathogenesis in 'Syndrome X' is more controversial (120) as no improvements in exercise capacity or ECG changes are seen

in this group after HRT (114). Because of the lack of improvement in objective measures in these women, it has been suggested that HRT may have primarily a symptomatic effect, with potential roles attributed to myocardial adenosine metabolism, a direct hormone-related analgesic effect or a central psychological effect (114).

The effects of both insulin resistance and oestrogen deficiency may go some way to explaining the observed common characteristics of those presenting with potential microvascular angina i.e. a high proportion of obese postmenopausal females. Although other factors may be at work, a 'double-hit' of oestrogen deficiency and insulin resistance may explain the observed bias towards these patients.

Figure 3:
Outline of the potential contributing factors to the abnormal vasomotor responses seen in some patients with cardiac 'Syndrome X'



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CHAPTER 3

Metformin in Insulin Resistance and Syndrome X (MIRS) Study:

Trial Design and Methods

Metformin in Insulin Resistance and Syndrome X (MIRS) Study

This double-blind randomised placebo-controlled study was undertaken at Glasgow Royal Infirmary between February 2000 and February 2002. There were 2 main components to the trial:

1. To compare women with cardiac 'Syndrome X' to healthy controls in terms of their metabolic parameters and peripheral microvascular function.

2. In women with cardiac 'Syndrome X' to investigate the changes in metabolic parameters, microvascular function and exercise test responses following 8 weeks treatment with metformin, compared to placebo.

Given the points discussed in the previous two chapters, the main general hypotheses were formulated:

- Coronary vascular dysfunction is an aetiological factor in microvascular angina

- Patients with microvascular angina have generalised vasomotor abnormalities in the systemic as well as coronary vascular beds.

- Resistance to the vascular effects of insulin is at least in part responsible for the vascular dysfunction observed in microvascular angina.

- Patients with microvascular angina, being relatively insulin-resistant, exhibit other features of the metabolic syndrome

- Taking a level of 2mm ST depression as threshold for a 'positive' exercise test will allow improved specificity of the ETT for ischaemia at the expense of sensitivity.

- Improving indices of insulin resistance by an insulin sensitising modality will improve vascular function and reduce symptoms in patients with microvascular angina.

The specific hypotheses we wanted to test in terms of this trial were:

- Women with 'Syndrome X' have different metabolic characteristics from age-matched control subjects, namely higher indices of insulin resistance and perhaps other features of the insulin resistance syndrome.

- If myocardial ischaemia in women with microvascular angina is caused by vasomotor disturbance leading to impaired vasodilator reserve, women with 'Syndrome X' will have impaired peripheral vascular function compared with age-matched healthy control subjects.

- Assuming insulin resistance has a causal role for vascular dysfunction, levels of insulin resistance should be correlated to peripheral endothelium-dependent microvascular function.

- 8 weeks of metformin therapy should improve levels of insulin sensitivity in women with microvascular angina and therefore vascular function. There is evidence that just 8 weeks of metformin is sufficient to demonstrate metabolic/endocrine effects in patients with polycytic ovaries. (1;2). This may lead to an improvement in symptoms in these women with 'Syndrome X'.

The study was carried out following the guidelines set out in the 'Declaration of Helsinki' and ethical approval for the study and its full protocol was obtained from the appropriate local authority.

Recruitment of Patients with 'Syndrome X'

The aim of recruitment was to identify women who fulfilled the criteria for 'Syndrome X' and invite them to participate. These were outlined in chapter two but in essence they consist of:

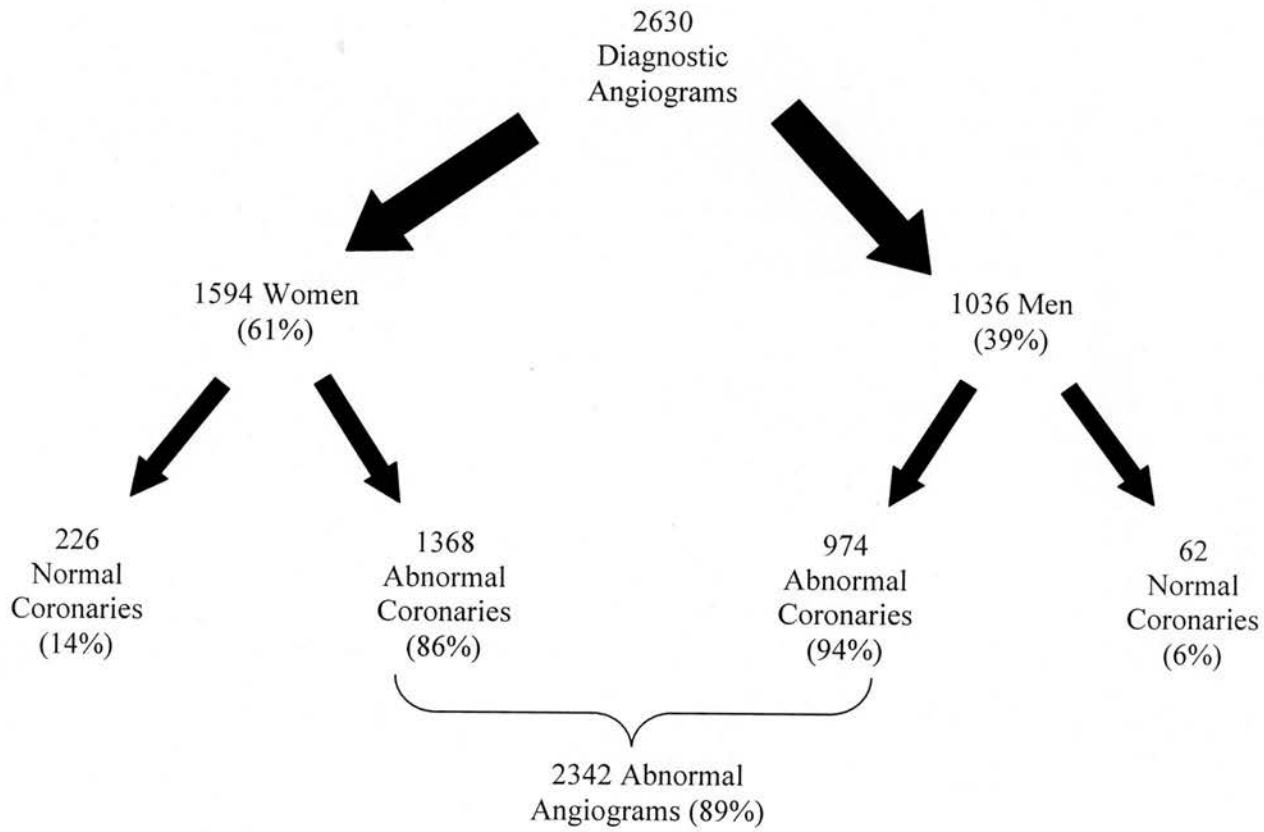
- Anginal-type chest pain
- Unobstructed epicardial arteries
- ECG changes during exercise typical of myocardial ischaemia

Coronary Angiography

I identified, retrospectively, all patients who underwent diagnostic coronary angiography in Glasgow Royal Infirmary between the period January 1998 and June 2001, on the basis of their angiogram reports as written by a consultant cardiologist. These were angiograms performed for the evaluation of ischaemic-sounding chest pain and all those patients undergoing angiography for other reasons (for example before valve surgery or to investigate left ventricular dysfunction) were excluded. All patients had been reviewed prior to angiography by a consultant cardiologist who had deemed symptoms sufficiently consistent with an ischaemic basis to warrant diagnostic coronary angiography. Potential recruits to the MIRS Study were those with smooth unobstructed epicardial arteries. Subjects with minor coronary atheroma were excluded as were those who had evidence of catheter-induced coronary spasm during angiography.

The flow chart below shows the numbers involved. As shown a total of 2630 coronary angiograms were performed during this period of 42 months. 288 of these identified smooth unobstructed coronary arteries and 226 of these subjects were female (78%). Surprisingly more women than men underwent diagnostic coronary angiography during this period (61% Vs 39%). However, the proportion of women in whom normal coronaries were found was significantly larger than the proportion of men (14% Vs 6%).

Figure 3.1:
Diagnostic Coronary Angiograms at
Glasgow Royal Infirmary Jan 1998- June 2001

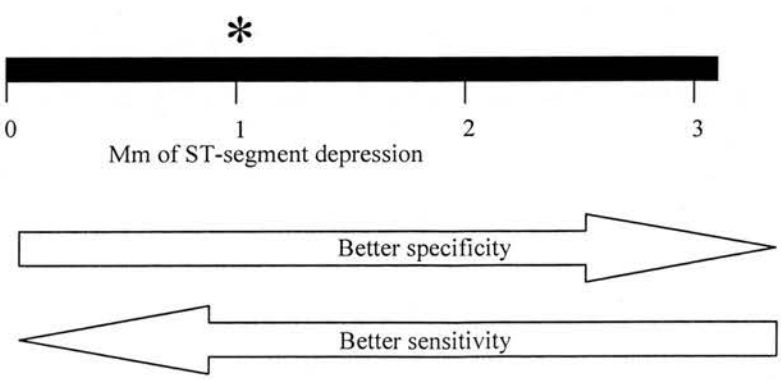


Electrocardiographic Criteria

I aimed to maximise the number with true microvascular angina and therefore genuine myocardial ischaemia, and keep to a minimum the subjects with non-cardiac pain. In practice this is very difficult to achieve given the specificity and sensitivity problems with the exercise tolerance test that have already been alluded to. In the past, studies which have applied strict electrocardiographic (ECG) criteria (>2mm ST depression) have proved specific and presumably this kept subjects with non-cardiac pain to a minimum. This is demonstrated by Boudoulas et al – they recruited in this manner and found that all of their 14 subjects exhibited abnormal lactate metabolism during pacing-induced tachycardia : an indicator of myocardial ischaemia (3). Although, this approach ensures specificity (very few subjects with non-cardiac pain will be included), it is at the expense of sensitivity (many subjects with genuine myocardial ischaemia will be excluded). Demonstrating this point, Mammohansingh et al used much less stringent ECG criteria (>0.5mm ST depression) and found that only 3 of this 14 recruits exhibited abnormal lactate metabolism under the same circumstances (4).

In order to achieve a balance between sensitivity and specificity I used a threshold level of 1mm of planar or down-sloping ST depression during exercise. Inevitably, this led to a number of patients with non-cardiac pain being recruited but was sensitive enough to identify sufficient numbers of individuals with myocardial ischaemia.

Figure 3.2:
Chart showing how the threshold taken for ST-segment depression affects specificity and sensitivity for myocardial ischaemia.

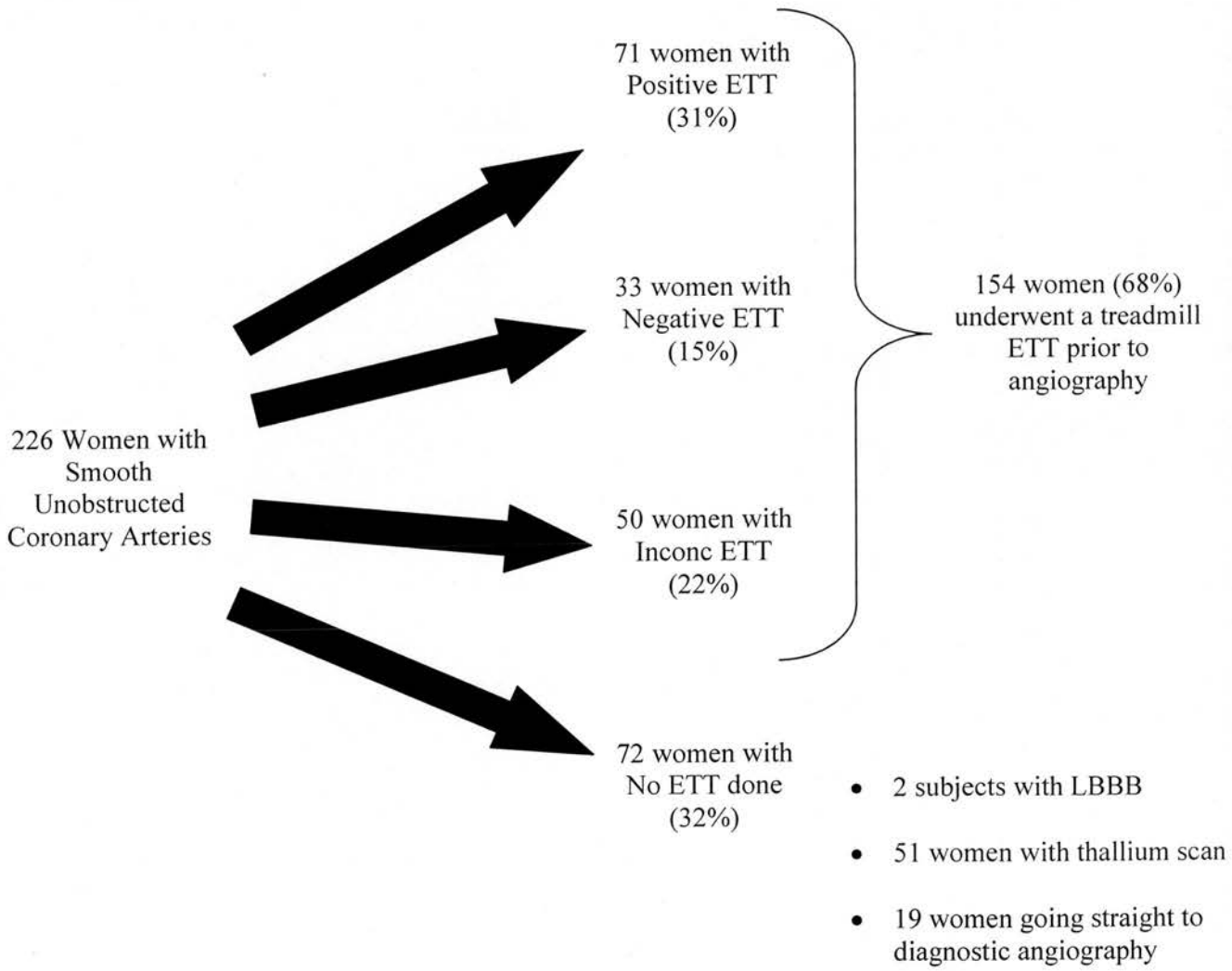


The casesheets of the 226 women with unobstructed smooth coronary arteries were pooled and the pre-angiogram exercise tolerance test result was examined. An ETT had been performed in 154 (68%) of women in whom subsequent angiography was normal. In 72 (32%) women in whom a treadmill ETT was not performed the vast majority had pre-angiogram thallium scintigraphy as an alternative non-invasive investigation, although in a significant number of women a diagnostic angiogram was sought without any prior non-invasive testing. These women generally had a very typical history for myocardial ischaemia with a significant risk factor profile.

Women who had thallium scintigraphy alone, without a standard treadmill ETT were not included. This was primarily to standardise the patients recruited and because ECG criteria rather than perfusion defects were used later in the study as the outcome measures.

Only 71 of the 154 women (46%) who underwent treadmill stress testing had a documented positive ETT. The stress ECG tracings were examined in all cases and a positive test was one in which at least 1mm of non-upsloping ST segment depression was seen during exercise. Of the remainder whom underwent treadmill testing 50 women (32%) had an inconclusive test in which either target heart rate was not achieved or only borderline ECG changes were documented. 33 women (21%) had a clearly negative ETT in which target heart rate was achieved without any significant ECG changes.

Figure 3.3:
Schematic outlining the pre-angiogram ETT result in the 226 women with normal coronary arteries.



Exclusion Criteria

Males

I chose to limit our patient group to females alone. Most investigators have described a female bias in 'Syndrome X' and this is reflected in my own experience, with 14% of women having normal coronaries compared to 6% of men. Numbers recruited therefore are likely to have a female bias. Hormonal factors may be important in microvascular angina, as discussed in chapter 2, and oestrogen deficiency in females has been reported within 'Syndrome X' (5). Although looking at hormonal influences was not one of the main objectives of the MIRS Study, characterising the study population in terms of menopausal status, HRT use and gynaecological history was deemed important, and the inclusion of what would likely have been a relatively small number of males in the study would confound these issues. In a randomised study of this size, inclusion of males may have resulted in potential male : female differences between the active group and the placebo group and the conclusions that I could draw in comparing the groups would have to take into account these differences. Therefore it was my intention to recruit females alone so that any differences seen in microvascular function and clinical parameters could be attributed to more easily to the observed metabolic changes rather than having to take hormonal/sex issues into account.

Type 2 Diabetes

Patients with type 2 diabetes were excluded because they are, by definition, insulin resistant. Therefore any conclusions about sub-clinical insulin resistance in women with chest pain and normal coronary arteries would be difficult and problems would be encountered by concomitant therapy such as other insulin-sensitisers and insulin itself. Impaired vascular function has been demonstrated in subjects with type 2 diabetes by a variety of methods including venous occlusion plethysmography (6), high resolution brachial artery ultrasound (7) and more recently laser doppler imaging (8). Therefore any inferences about vascular function in this patient group would be

confounded by patients with known type 2 diabetes. Patients were, therefore, excluded on the basis of a pre-existing diagnosis of type 2 diabetes documented in the casesheet or by means of a fasting plasma glucose <7.0mmol/L (World Health Organisation criteria) taken during an assessment visit.

Hypertension

Patients with existing uncontrolled hypertension (blood pressure >150/90 mmHg) were excluded from the study on the basis of data demonstrating abnormal vascular responses in subjects with hypertension, as assessed by both plethysmography (9) and high resolution brachial artery ultrasound (10). All subjects underwent transthoracic echocardiography and were excluded if significant left ventricular hypertrophy was seen (interventricular septum or posterior wall thickness >12mm). Normotensive subjects without echocardiographic evidence of left ventricular hypertrophy were included in the study.

Table 3.1:
Table summarising the exclusion criteria for this trial

	Exclusion Criteria
Patients fulfilling criteria for cardiac 'Syndrome X'	Males
	Diabetes Mellitus
	Uncontrolled hypertension or left ventricular hypertrophy on ECHO (150/90 mmHg)
	Significantly abnormal resting ECG (incl LBBB*)
	Structurally abnormal heart
	Renal impairment (creatinine > 130μmol/L
	Hepatic impairment (transaminases > x2 upper limit of normal)
	Previous history of lactic acidosis

* left bundle branch block

Other Criteria

Patients with a significantly abnormal resting electrocardiogram (including left bundle branch block) were excluded. This was primarily because ST-segment changes during exercise were an important piece of data and this is uninterpretable in patients with a grossly abnormal resting ECG. Those with other potential cardiac contributors to chest pain were not recruited. This included those with cardiomyopathy and any degree of valvular heart disease identified either during casesheet inspection or during the baseline echocardiogram.

Those subjects with abnormal renal or liver function were also excluded. This was because these patients could potentially be commenced on metformin during the treatment period. As renal impairment and abnormal liver function are contra-indications to metformin therapy, these patients were not included. The definition used were :

Abnormal renal function - serum creatinine >130mmol/L

Abnormal liver function - AST or ALT > twice the upper limit of normal

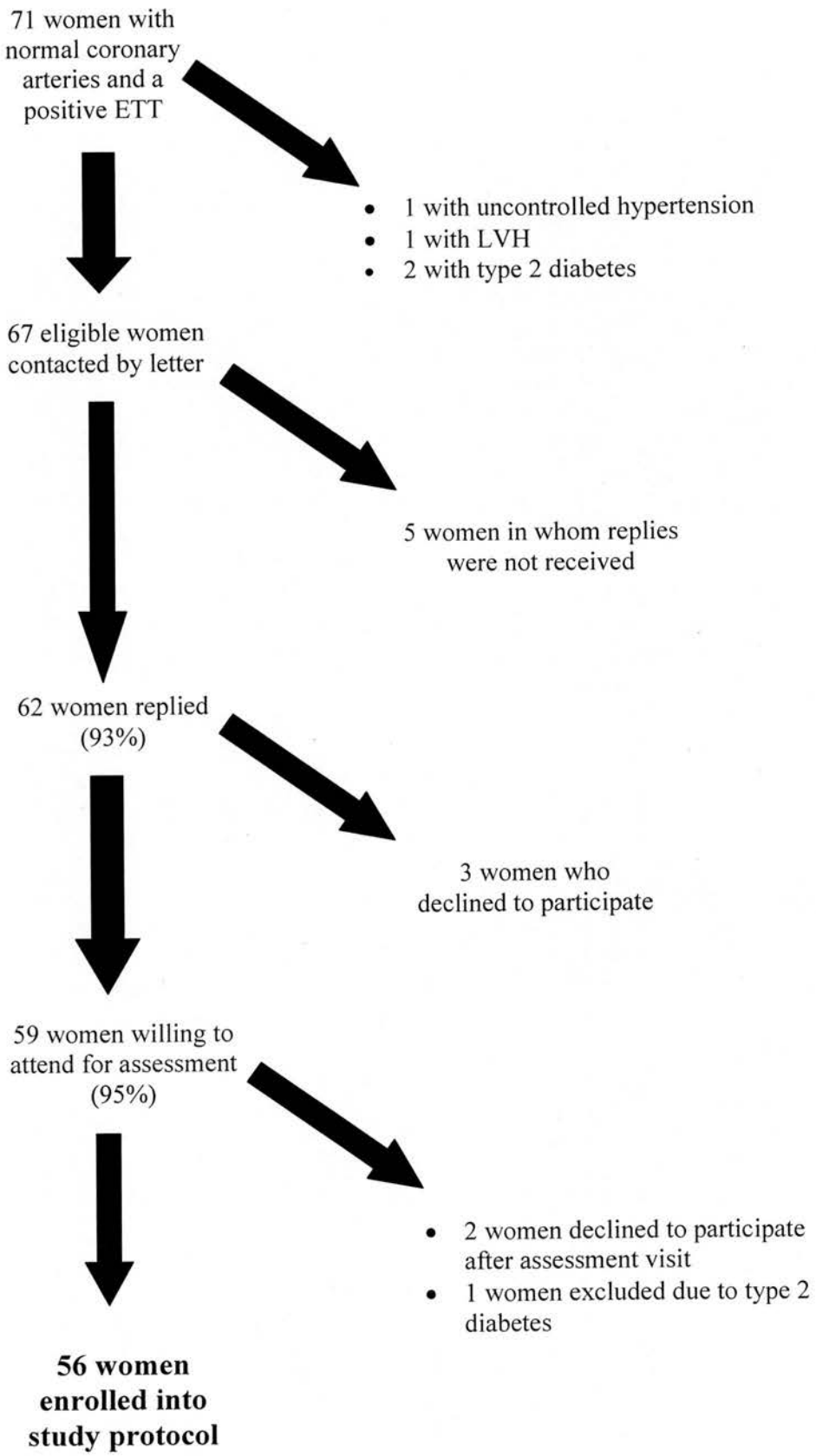
Cigarette smokers were not excluded.

Final Numbers

On the basis of the recruitment protocol 71 women were identified as suitable candidates for inclusion in the MIRS Study, having had a positive ETT and unobstructed coronary arteries on angiography (see figure 4). These 71 women were examined for exclusion criteria discussed. Only 2 women out of these 71 subjects had significant hypertension with one subject having documented left ventricular hypertrophy on echocardiography. 2 subjects had type 2 diabetes. No subjects with significant valve disease were identified. These 4 subjects were excluded leaving a total of 67 suitable candidates for participation in the MIRS Study.

All 67 of these women were written to with details of the proposed study and a stamped-addressed envelope was provided for reply to indicate whether they would be willing to attend for a screening visit to Glasgow Royal Infirmary. Replies were obtained from 62 women (93%) and all but 3 were willing to attend for the assessment visit. At the assessment visit 2 women did not want to participate further and 1 was excluded due to a high fasting blood glucose. No patients were excluded on the basis of abnormal liver or renal function. This left 56 women who were entered into the study protocol which is described in the next section (see figure 4).

Figure 4:
Schematic outlining recruitment methods



Study Protocol

As can be seen from the above schematic 59 women, in whom smooth unobstructed coronary arteries and a positive ETT were recorded, accepted the invitation to attend Glasgow Royal Infirmary for an assessment visit. The purpose of this was to provide patients with information about the main trial, to make some baseline measurements, to take some history and make a final decision about eligibility for the study.

Assessment Visit

Patients were contacted by telephone and arrangements were made for them to attend the Royal Infirmary. During this visit which lasted approximately 30 minutes several objectives were accomplished:-

- History of chest pain character and frequency was documented
- Other history including co-morbid conditions, drugs, family history and gynaecological history
- Blood pressure measurement
- Fasting blood glucose and hormone profile (FSH, LH and oestradiol)
- Blood taken for liver function and urea and electrolytes
- Brief explanation of the study background and objectives
- If willing to take part and eligible – informed consent was obtained at this stage (consent form in appendix 1)

- Written information about the study was provided both to the patient and the general practitioner (copies shown appendix 2)

- Anti-anginals were minimised and discontinued for the majority of patients where possible (see table 2). As can be seen, the vast majority of vasoactive / metabolically active drugs were discontinued. Aspirin, cholesterol-lowering therapy and HRT were not changed.

- Arrangements were made for the first baseline visit in 4 weeks time with instructions asking patients to fast from midnight the night before

- Patients provided with a chest pain chart to document frequency of anginal pain

The general practitioner was informed of the patient's participation in the study along with some background information regarding the study objectives. Any treatment which had been suspended for the purposes of the study was also disclosed. A copy of this letter was placed in the patient's hospital casesheet.

Table 3.2: Medication at assessment and during study (including 4 week wash-out period)

Drugs	*Assessment*	*During study*
Aspirin	41 (73%)	41 (73%)
Beta-Blockers	16 (29%)	1 (2%)
Amlodipine	7 (13%)	1 (2%)
Diltiazem	19 (34%)	3 (5%)
Nitrates	14 (25%)	5 (9%)
Nicorandil	5 (9%)	3 (5%)
Statin	22 (39%)	22 (39%)
HRT	14 (25%)	14 (25%)

Baseline Investigation Visit

Patients were met at the Cardiology department at 8:30am on the day of the baseline visit. Fasting overnight was confirmed. After this a series of procedures was undertaken which took most of the morning to complete. At 1pm subjects were permitted to have a light lunch prior to the treadmill test. All other investigations were completed before this. Procedures undertaken during the morning :

- Trans-thoracic echocardiogram

- Assessment of peripheral endothelial function using a laser Doppler imager to document changes in forearm cutaneous perfusion in response to incremental doses of topically applied sodium nitroprusside (SNP) and acetyl choline (ACh). These vasoactive drugs were delivered non-invasively by iontophoresis. The method is expanded upon in chapter 5.

- Fasting blood tests after the endothelial function tests. These included :

  - glucose and insulin
  - free fatty acids
  - lipid beta quantification
  - C-reactive protein
  - leptin
  - endothelial serum markers (von Willebrand factor (vWF), intracellular cell adhesion molecule (I-CAM), vascular cell adhesion molecule (V-CAM), tissue plasminogen activator (tPA), and D-dimer
  - lactate

- Oral glucose tolerance test with blood collected for measurements of glucose, insulin and free fatty acids at 60 minutes and 120 minutes.

- Blood pressure recordings x3 with an average recorded

- Character of chest pain assessed with objective questionnaire and frequency of chest pain and GTN use derived from chest pain chart.

- Treadmill exercise test using the full Bruce Protocol

Chest Pain Assessment

Frequency of chest pain was judged by the chest pain chart which was completed during the 4 week run-in period prior to the baseline visit. The 'Daily Pain Index' was calculated by adding up the total number of episodes of chest pain and dividing by the number of days during which recording was made. A similar 'Daily GTN Index' was calculated by summing the number of times GTN was used and dividing by the number of days during which recording was made. The Canadian Cardiac Society (CCS) index of angina severity was also recorded. This numerical index ranges from 1 (minimal angina at maximal exertion) – 4(severe angina at rest). At the end of this visit patients were supplied with a further chest pain chart to document symptoms over the 8 week treatment period and a date for follow-up investigations decided.

Administration of Metformin/Placebo

Packs were dispensed from pharmacy at Glasgow Royal Infirmary and given to patients with instructions at the end of day 1 investigations. The study was double-blinded and packs of metformin and placebo were randomised in using a 2x2 method. The metformin and identical placebo were supplied by Merck and packaged by Pharmacy at North Glasgow University NHS Trust. The metformin packs were numbered 1-60 and patient names recorded against the appropriate number dispensed. The randomisation codes were held by pharmacy throughout the study and were only made available after all the data had been collected and analysed.

The dose of metformin used was 500mg once daily for 1 week building up to 500mg twice daily thereafter for the remainder 7 weeks of treatment. This system was used to introduce metformin gradually and minimise side-effects. 130 tablets were dispensed to each patient with written instructions. Patients were warned about potential side effects especially gastro-intestinal disturbance and reassured that they ought to persevere with the study medication as the majority of these side-effects are transient. Patients were, however, asked to record any side-effects.

Patients were asked not to start any new or discontinue any existing therapies during the study period. Compliance with metformin/placebo was checked at the end of the study by means of pill-counting. 8 weeks of therapy using this regime ought to require 105 tablets and therefore 25 tablets should remain at the end of the treatment period assuming all doses were administered and no wastage occurred.

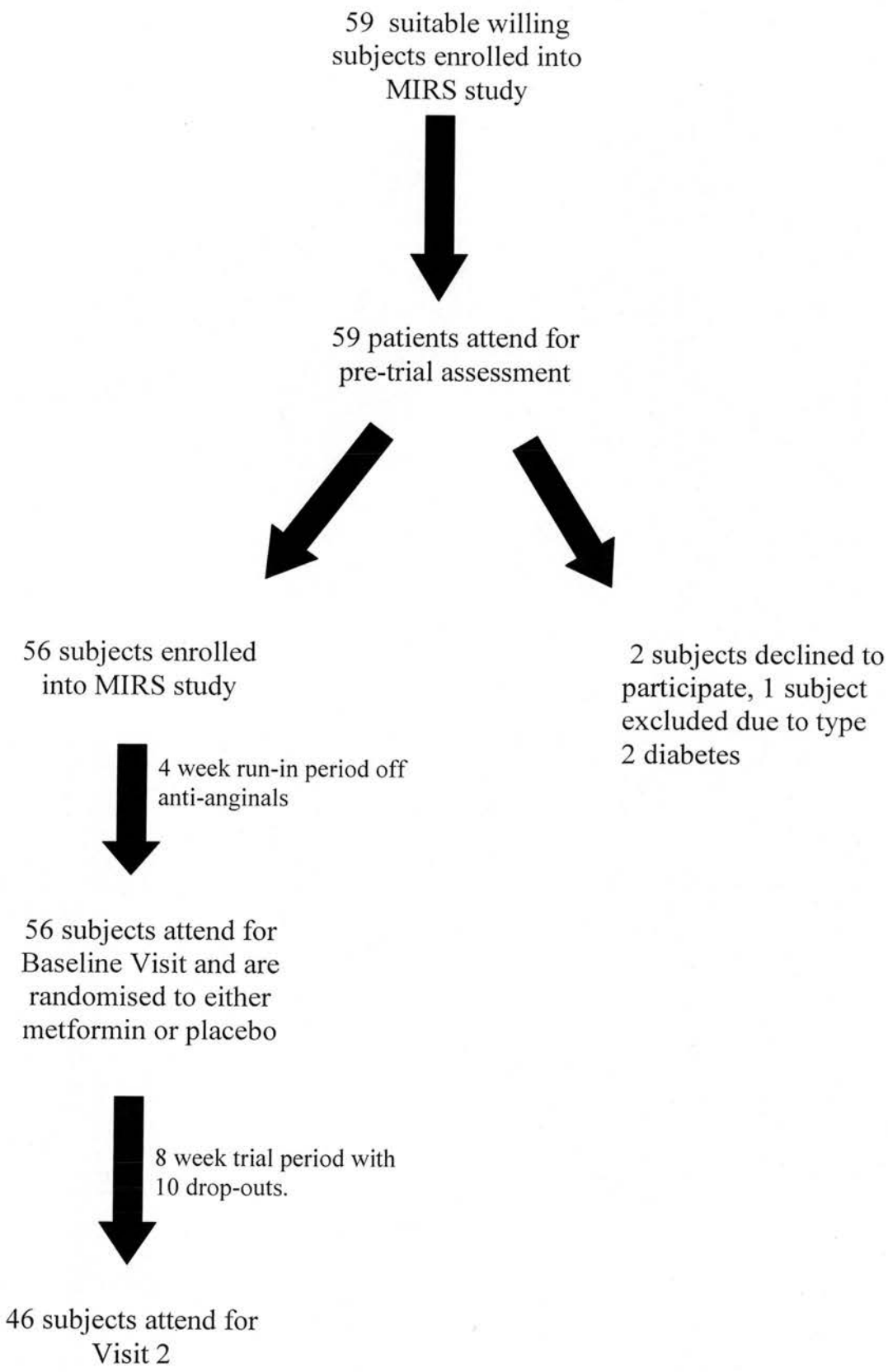
Follow-up Investigation Visit

The same investigations were performed in the follow-up visit after 8 weeks of treatment with either placebo or metformin, with the exception of the echocardiogram. The CCS angina index, the daily chest pain index and the daily GTN-use index was also calculated for each patient during the 8 week treatment period as described for the baseline visit. The remainder of the tablets were collected and the number recorded. The patients were recommenced on their pre-study medication, although in a significant number of individuals a clinical decision was made not to do this, because of a lack of any symptomatic difference on and off treatment.

In all cases the general practitioner was contacted and the results of fasting glucose, cholesterol, blood pressure measurements and treadmill mill test performance was conveyed. The treatment regime post-study was also recorded in this letter, along with any other recommendations regarding blood pressure therapy, cholesterol-lowering agents and treatment for ongoing chest pain.

At the end of the study patients were discharged from follow-up except in a few cases where follow-up was required for specific reasons such as blood pressure monitoring or excessive ongoing symptoms. Routine outpatient follow-up was arranged at the general cardiology clinic at Glasgow Royal Infirmary for these individuals.

Figure 3.5:
Flowchart to illustrate the
MIRS study patient visits



Drop-outs From Study Protocol

As can be seen from the flow-chart (figure 5), 56 women entered the study protocol, attended for baseline investigations and were dispensed either metformin or placebo. As is inevitable with clinical research, not all of these 56 women completed the full course of treatment for 8 weeks.

There were 10 patients who did not complete the study protocol and therefore did not have a set of follow-up investigations performed. The reasons for drop-out are varied and listed below:-

- 2 patients uncontactable through home address, telephone number and G.P.

- 4 patients who stopped treatment because of side-effects, all of which were gastro-intestinal

- 1 patient was admitted to hospital with a prolonged episode of chest pain and the study medication was discontinued by the admitting physician

- 2 stopped the medication themselves because of ongoing non-specific symptoms during the treatment period which pre-dated the study

- 1 patient stopped the treatment because she felt unwell during an upper respiratory tract infection and did not restart

Recruitment of Control Subjects

It was my intention to make comparisons between data collected on women with 'Syndrome X' and normal healthy controls. To this end I aimed to recruit between 20-30 control subjects. This was done by placing an advertisement in the Glasgow University newsletter outlining the study and asking for volunteers to come forward. The inclusion criteria were:

- Women aged between 40-65 years
- Peri-menopausal or postmenopausal
- No history of chest pain or any other cardiac problem
- No history of diabetes or hypertension

By the use of advertisement 25 women came forward and were able to attend Glasgow Royal Infirmary for the study investigations. The protocol for the control subjects was much simpler than for the women with 'Syndrome X'.

Control Subject Study Protocol

Subjects were requested to attend the Royal Infirmary fasting in the morning. In this state the following procedures were performed:

- Brief history was obtained to document personal and family history of heart disease, diabetes and blood pressure as well as menopause status.

- Assessment of peripheral endothelial function using a laser Doppler imager to document changes in forearm perfusion in response to incremental doses of topically applied sodium nitroprusside (SNP) and acetyl choline (ACh). These vasoactive drugs were delivered non-invasively by iontophoresis.

- Fasting blood tests after the endothelial function tests were completed.

  - glucose and insulin
  - free fatty acids
  - lipid beta quantification
  - C-reactive protein
  - leptin
  - endothelial serum markers (von Willebrand factor (vWF), intracellular cell adhesion molecule (I-CAM), vascular cell adhesion molecule (V-CAM), tissue plasminogen activator (tPA), and D-dimer
  - lactate

Control subjects did not have an exercise tolerance test.

Methods

Clinical Symptom Parameters

Assessment of chest pain frequency and the effect of metformin was an important outcome on which the MIRS trial focused. By definition this is a very subjective measure and therefore, attempts to objectify it as much as possible were put in place. Making this measurement as objective as possible makes comparisons between treatment groups feasible.

Patients were supplied with a chart which they used as a 'chest pain diary'. They recorded on this chart every significant episode of chest pain that they experienced. They also recorded the number of times they used their glyceryl trinitrate (GTN) spray. By this means, a 'chest pain index' and a 'GTN index' could be calculated by dividing the number of episodes recorded by the total number of days of the chart duration. Therefore a chest pain or GTN index of 1.0 implies an average of one episode of chest or GTN use per day, during the period examined. A separate chart was given to each patient for both the 4 week run-in period prior to the baseline visit, and the 8 week period of the study, prior to visit 2.

Chest pain frequency and severity was also assessed using the Canadian Cardiovascular Society (CCS) Index. This is an index numbered 0-4 which is applied as below:

Class 0: Asymptomatic

Class 1: Angina with strenuous Exercise

Class 2: Angina with moderate exertion

Class 3: Angina with mild exertion

- Walking 1-2 level blocks at normal pace
- Climbing 1 flight of stairs at normal pace

Class 4: Angina at any level of physical exertion

Blood Pressure Measurements

Blood pressure was measured on the right arm by a cuff sphygmanometer. An automated digital 'Omron' machine was used to keep measurements objective. Measurements were taken in the supine position before venepuncture. A total of 3 measurements were taken during each visit and an average was used to represent the blood pressure during that visit.

Anthropometric Measurements

Measurements of height and weight were taken to calculate body mass index during each visit. Height was measured by a ruler scale fixed to a wall to avoid inter-visit variation. Weight was measured by a 'Seca' digital scale, with footwear removed. Body mass index was then calculated by the standard formula :

Weight in kg / (height in metres) ^{2}

Waist and hip circumference were measured manually by a standard tape measurement. Waist circumference was taken at the level of the umbilicus. Hip measurement was taken at the maximal point around the hip. Waist : Hip ratio was then calculated.

Blood Tests

Sampling

In all cases and controls blood tests were obtained in the fasting state after the microvascular function tests were completed. This was to ensure that the minor stress associated with venepuncture did not interfere with these other tests. In the cases of women with 'Syndrome X', an 18-guage intravenous cannula (venflon) was inserted into the right antecubital fossa to facilitate further blood sampling during the oral glucose tolerance test. With the control subjects, blood was collected using the 'vacutainer' system. The venous blood was centrifuged immediately after collection at 3000rpm for 10 minutes to separate out the serum and the serum was pipetted into 2ml alloquots for immediate freezing and storage at -80^{0}C until batch analysis which was undertaken at the of the study.

An oral glucose tolerance test (OGTT) was performed in the subjects with 'Syndrome X'. After insertion on the intravenous cannula, and the extraction of a fasting blood sample, the venflon was flushed with saline to keep it patent. At time 0' 75g of glucose was administered orally. This took the form of 415ml of lucozade which was drunk within a 2 minute period.

At time 60' and 120 minutes, blood was withdrawn from the indwelling venous cannula for glucose, insulin and free fatty acids, after discarding the initial 5ml of saline-contaminated blood. After the sample was obtained, the cannula was again flushed with saline. The cannula was removed immediately after the last sample was obtained. The samples obtained during the oral glucose tolerance test were treated in the same way as the fasting blood samples.

- Insulin and Glucose

The plasma glucose measurement is presented in mmol/L.

Hyperinsulinaemia is used as a surrogate marker of insulin sensitivity. Data are presented on fasting insulin in mu/L. These data are log transformed in order to achieve a normal distribution and allow comparison by means of standard statistical tests. The Quicki index is also presented. This is outlined as a measure of insulin sensitivity in chapter 1. It incorporates both fasting glucose and fasting insulin measures as well as a log and reciprocal transformation to restore normality to the distribution. It is calculated thus:

Quicki index = (1/[(log insulin) + (log glucose)]

Although the gold-standard for measuring insulin resistance is the euglycaemic clamp method as outlined in chapter 1, I made use of fasting insulin because performing euglycaemic clamps on all subjects in a study of this size is very labour intensive. Moreover, others have demonstrated good agreement between fasting insulin and clamp-derived indices of insulin resistance, especially in obese populations (11).

Non-esterified fatty acids (NEFA) are also presented and as these are normally distributed, they are shown directly.

- Lipids

A full lipid profile is presented. Serum total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol and triglyceride are all measured in mmol/L. The three cholesterol parameters are all normally distributed and are therefore presented directly. However, serum triglyceride is not normally distributed and these data are presented as a reciprocal transformation to achieve normality.

- Serum Endothelial Markers

Various measures are presented including tissue plasminogen activator (t-PA), vascular cell adhesion molecule (V-CAM), intercellular adhesion molecule (I-CAM), D-dimer and von Willebrand factor (vWF). All of these data require a log transformation to achieve a normal distribution, except t-PA and are thus presented as such. t-PA follows a normal distribution and is presented directly.
t-PA, I-CAM and V-CAM are measured in ng/L, vWF is expressed as a % and d-dimer is measured in arbitrary units.

- C-reactive Protein

A high sensitivity C-reactive protein (CRP) was measured as a marker of inflammation. These data are measured in mg/L and require a log transformation in order to follow a normal distribution and are therefore presented as such.

- Leptin

This is a serum marker related to adiposity and is measured in ng/L. These data follow a normal distribution and are therefore presented directly.

Exercise Tolerance Test

This was done in the subjects with 'syndrome x' both during the baseline and the follow-up visit. It was performed at the end of the visit and subjects were allowed to have a light lunch before embarking on this test. A 'Marquette' treadmill was used with a computerised controller. All patients were subjected to a full Bruce Protocol. Exercise was symptom-limited. Standard measurements were recorded including:

- Limiting symptom and the occurrence of anginal chest pain
- Total exercise time
- Maximal ST-segment deviation (calculated manually using pencil and ruler from hard copy ECG to nearest 0.5mm. This prevents artefactual interference which would contaminate result if the automatically calculated, average computer-generated ST-segment deviation were used)
- Duke Score

The Duke Score is a numerical score ranging from –25 to +10 reflecting the performance of the patient during the treadmill test. It is calculated as following :-

Total Exercise Time in Minutes	-	4 x Maximal ST Segment Depression in mm	-	5 x Pain Index

Pain Index :
0 : no pain during exercise
1 : non-limiting pain during exercise
2 : limiting pain during exercise

It is a useful index to describe overall performance during the exercise tolerance test. It has been used in patients with coronary disease as a prognostic indicator (12).

Assays

All blood samples were frozen in aliquots and stored at -80°C. They were all analysed at the end of the study in batches before the randomisation codes for metformin and placebo were broken.

Samples were analysed quantitatively for insulin using a Microparticle Enzyme Immunoassay (MEIA) - IMx® from Abbott Laboratories. The coefficient of variation as <8% and it had a sensitivity of 0.8mU/l.

Plasma glucose was measured using the standard glucose oxidase method in the laboratories of Glasgow Royal Infirmary. (Glucose Reagent Kit - Olympus AU5200, Olympus Optical Co Ltd)

Lipid profile was measured using established methods (Boehringer reagent kits, East Sussex, UK) in a laboratory meeting CDC standardisation criteria.

Tissue plasminogen activator (t-PA), D-dimer and von Willebrand factor (vWF) were measured by ELISA techniques. In the case of t-PA and D-dimer the ELISA was from Biopool (Stockholm, Sweden) and for vWF the ELISA was from Dako (Copenhagen, Denmark).

Vascular cell adhesion molecule (V-CAM) and intercellular adhesion molecule (I-CAM) were assayed using commercially-available ELISAs quantification (R&D systems Inc., Oxon UK)

C-reactive protein (CRP) was measured using a double antibody sandwich ELISA with rabbit anti-human CRP and peroxidase conjugated rabbit anti-human CRP (DAKO A/S, DK-2600 Glostrup, Denmark). Standard curves for CRP measurement were linear up to 5mg/l and thereafter logarithmic. The inter-assay and intra-assay coefficients of variation were less than 7% across the range of measured results. The lower detection limit of this assay was 0.1mg/l.

Leptin was measured by an in-house radioimmunoassay that has been validated thoroughly against the commercially available Linco assay. The intra-assay and interassay coefficients of variation were less that 7% and <10% respectively over the sample concentration range. The detection limit for the assay was 0.5ng/ml.

Laser Doppler Imaging

Peripheral endothelium-dependent and endothelium-independent microvascular function was assessed using laser Doppler imaging of the forearm cutaneous vascular bed with the subject relaxed in the supine position in a quiet, temperature and light-controlled environment. This was carried out before any blood tests were obtained. The microvascular response to acetyl choline (ACh) and sodium nitroprusside (SNP) was sought and these vasoactive drugs were delivered to the forearm cutaneous bed non-invasively by iontophoresis.

Perspex iontophoresis chambers were applied to the extensor forearm surface. The cathode chamber was filled with a 1% sodium nitroprusside (SNP) solution (dissolved in 0.5% sodium chloride) and the anode chamber 1% acetyl choline (ACh) solution (also dissolved in 0.5% sodium chloride). Incremental current was applied with concurrent laser Doppler imaging to record the perfusion response. The laser Doppler imager (Moor Instruments Ltd) made use of a red laser (wavelength 633nm, power 1mW, beam diameter 1mm). The voltages across the circuit were recorded to enable calculation of the skin resistance as previously described. The perfusion response in flux perfusion units was calculated using Moor Instruments software package and is presented as both area under the curve for the scan and as a plot of flux perfusion units against cumulative charge. The mean ($\pm$ SD) between-day coefficient of variation in healthy subjects for the ACh response was 6.4 $\pm$ 3.3% whilst the within-day, between-site coefficient of variation, measured in both forearms was 8.9 $\pm$ 5.3%.

This novel method for assessing microvascular function will be the subject of chapter 5 and a more detailed description of the method employed will be given at this point.

Statistics

The statistical methods employed to compare the differences in the measured variables at various stages are described in detail in the relevant chapters. A brief outline is given below:

- Variable distributions were tested for normality and where necessary appropriate transformations were made so that parametric statistical comparison tests could be employed.

- Continuous variables were compared by the unpaired t test for differences between the Syndrome X group and control group at baseline

- Continuous variables were compared by the paired t test for differences between the placebo and metformin groups (Syndrome X patients only)

- Dichotomous variables were compared by the chi-squared test where appropriate

- The effects of differences (in age and BMI) between the Syndrome X group and healthy controls were adjusted by regression analysis

- Correlations between measured variables were looked at Pearson's correlation co-efficient

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CHAPTER 4

Assessment at Baseline and Comparisons Between Healthy Controls and Women with 'Syndrome X'

Comparisons Between Healthy Female Controls and Women with Syndrome X

This chapter looks at the physical data collected at baseline in the cohort of 56 women with cardiac 'Syndrome X'. Comparisons are made with the 25 healthy controls subjects in most cases to establish whether women with cardiac 'Syndrome X'exhibit any specific characteristics.

Statistics

Distribution

Most of the variables within the 2 groups were normally distributed. This was tested using frequency histogram plots as well as the more objective Shapiro-Wilk test. Some variables did not quite meet the stringent normality criteria of this test. These variables include diastolic blood pressure in the healthy controls, HDL-cholesterol in the Syndrome X group, von Willebrand factor (vWF) in the Syndrome X group, non-esterified fatty acids (NEFA) in the healthy control group, waist hip ratio and body mass index in the Syndrome X group and leptin in the healthy control group. The normality plots of these variables tended towards a gaussian distribution but failed to satisfy the strict criteria of the Shapiro-Wilk. It is known, however, that these variables are distributed normally in large scale populations and it is probable that they did not satisfy the Shapiro-Wilk criteria due to the relatively small sample size and the effect of outliers. For these variables a normal distribution was assumed as this was suggested by the frequency histogram plot.

However, some other variables did not tend towards a normal distribution at all and these include triglycerides, von Willebrand factor (vWF), d-dimers, I-CAM and V-CAM, insulins, and CRP of both groups. These distribution data are summarised in table 1 below.

Table 4.1:
Table illustrating the distribution pattern of the variables measured.

Normal		**Tending towards normality**		**Not normally distributed**	
Age	(both groups)	Diastolic Bp	(controls)	Triglycerides	(both groups)
Systolic Bp	(both groups)	HDL Chol	(syndrome x)	vWF	(both groups)
Diastolic Bp	(syndrome x)	NEFA	(controls)	D-dimer	(both groups)
Total Chol	(both groups)	W:H ratio	(syndrome x)	ICAM	(both groups)
LDL Chol	(both groups)	BMI	(syndrome x)	VCAM	(both groups)
HDL Chol	(controls)	Leptin	(controls)	Insulin	(both groups)
tPA	(both groups)			CRP	(both groups)
Glucose	(both groups)				
NEFA	(syndrome x)				
W:H ratio	(controls)				
BMI	(controls)				
Leptin	(syndrome x)				

In order for parametric tests to be applied, the variables which failed to satisfy tests for normality underwent transformations. These are listed below. The transformed variables did satisfy normality criteria by the Shapiro-Wilk test.

- Triglycerides — reciprocal transformation
- Von Willebrand Factor — log transformation
- D-dimers — log transformation
- I-CAM — log transformation
- V-CAM — log transformation
- Insulin Sensitivity — quicki index (defined in chapter 3)
- Serum insulin — log transformation
- C-reactive protein — log transformation

Variance

The groups of data collected in both the control subjects and those with Syndrome X were compared against each other to test for equal variance using Levene's test. The variance is a measure of how spread out a distribution is. It is computed as the average squared deviation of each number from its mean. Variance was equal in all groups of data and this is of importance when applying statistical tests looking for differences between the groups.

Presentation of Data

Parametric statistical tests make the assumption that data are normally distributed. This is not the case with all of the data collected. Therefore, the analysis was undertaken on normally distributed variables and on the normally distributed transformations of the non-normally distributed variables listed above.

The raw data for each set of variables is presented in tabular format with patients being identified by an ID code to preserve confidentiality. The data sets are therefore subsequently described in terms of the mean and standard deviation. The 2 sample t-test has been used to look for differences between the data sets for each variable of interest. Statistical analysis was done using a computer and the package Minitab® Statistical Software.

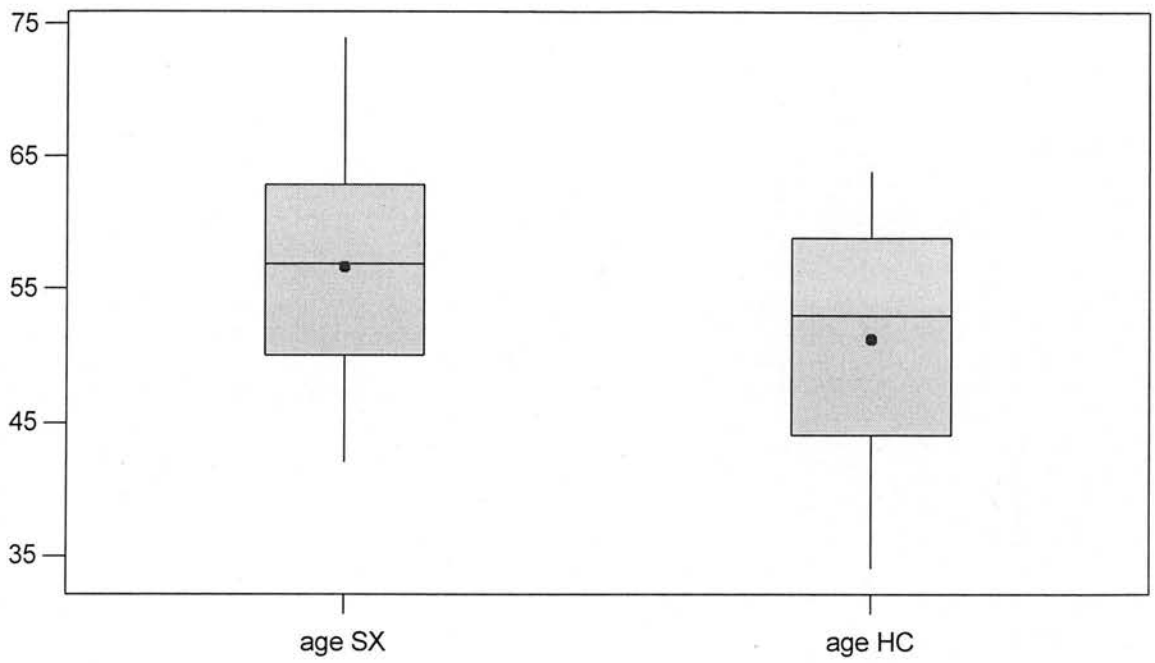
The boxplots for each data set are shown with the central line representing the mean and the box edges the standard deviation. The whiskers demonstrate the entire range of the data with outliers being shown as a cross.

1: Age

56 patients with Syndrome X and 25 healthy controls listed with DOB and age on 31/12/01

Syndrome X *Healthy Controls*

Patient ID		Age (yrs)		Control ID		Age (yrs)
SX001		54		HC001		55
SX002		61		HC002		51
SX003		66		HC003		53
SX004		43		HC004		41
SX005		60		HC005		40
SX006		43		HC006		44
SX007		55		HC007		61
SX008		74		HC008		64
SX009		63		HC009		53
SX010		53		HC010		50
SX011		49		HC011		46
SX012		57		HC012		46
SX013		65		HC013		48
SX014		60		HC014		53
SX015		70		HC015		61
SX016		55		HC016		55
SX017		68		HC017		62
SX018		50		HC018		61
SX019		55		HC019		57
SX020		52		HC020		62
SX021		42		HC021		54
SX022		59		HC022		44
SX023		72		HC023		44
SX024		51		HC024		43
SX025		64		HC025		34
SX026		57				
SX027		61				
SX028		63				
SX029		60				
SX030		60				
SX031		71				
SX032		53				
SX033		57				
SX034		57				
SX035		61				
SX036		65				
SX037		45				
SX038		63				
SX039		46				
SX040		62				
SX041		64				
SX042		55				
SX043		69				
SX044		69				
SX045		45				
SX046		43				
SX047		49				
SX048		48				
SX049		49				
SX050		46				
SX051		52				
SX052		61				
SX053		50				
SX054		56				
SX055		51				
SX056		48				
Mean		**56.7**		**Mean**		**51.3**



Two-Sample T-Test: Age

Group	n	Mean (years)	Range	Standard Deviation	Standard Error of Mean
Syndrome X	56	56.73	42-74	8.32	1.1
Controls	25	51.28	34-64	8.11	1.6

Mean Difference (years)	5.45	
95% Confidence interval for difference	lower	higher
	+1.50	+9.41
p value	**0.008**	

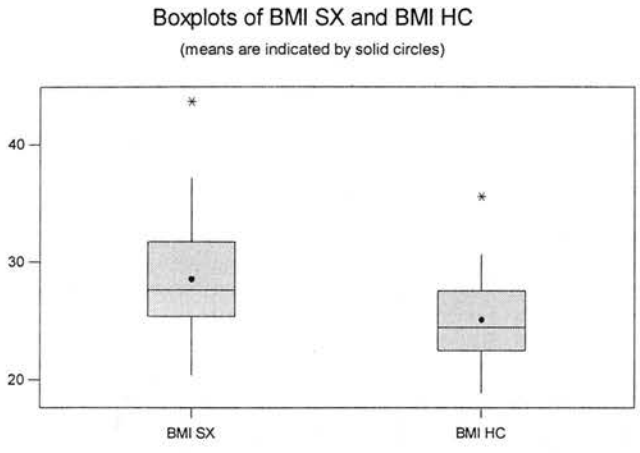
There is therefore a significant difference between the ages of the subjects in the 2 groups. The subjects in the Syndrome X group are significantly older by an average of almost 5.5 years.

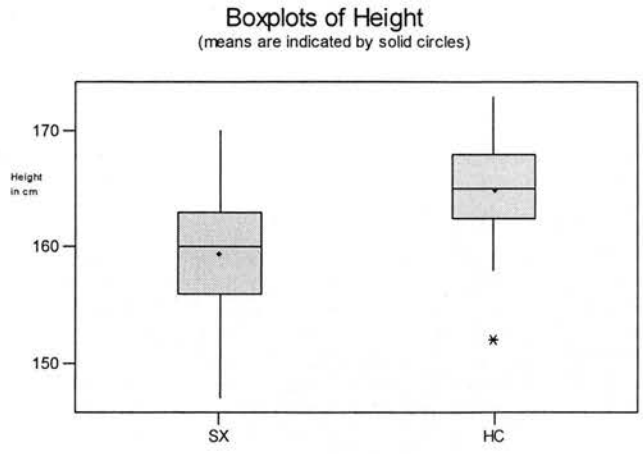
2: Body Mass Index

56 patients with Syndrome X listed with height and weight results at baseline along with 25 healthy controls.

Syndrome X *Healthy Controls*

Patient id	Ht (cm)	Wt (kg)	BMI (kg/m^2)		Control id	Ht (cm)	Wt (kg)	BMI (kg/m^2)
SX001	157	74	29.8		HC001	163	67	25.0
SX002	158	66	26.4		HC002	167	77	27.7
SX003	157	62	25.0		HC003	170	67	23.3
SX004	148	82	37.2		HC004	166	72	26.4
SX005	164	69	25.7		HC005	166	67	24.5
SX006	161	53	20.4		HC006	165	74	27.0
SX007	158	59	23.6		HC007	159	60	23.9
SX008	154	59	24.9		HC008	152	69	30.1
SX009	165	81	29.6		HC009	166	66	23.8
SX010	147	70	32.2		HC010	173	63	21.0
SX011	160	85	33.2		HC011	170	63	21.8
SX012	159	55	21.6		HC012	171	82	28.0
SX013	158	76	30.4		HC013	172	76	25.7
SX014	158	87	34.9		HC014	162	56	21.1
SX015	153	69	29.6		HC015	164	52	19.1
SX016	163	65	24.5		HC016	165	60	22.0
SX017	156	69	28.4		HC017	158	61	24.2
SX018	153	80	34.2		HC018	163	63	23.5
SX019	154	58	24.5		HC019	160	59	23.0
SX020	165	70	25.5		HC020	165	52	18.9
SX021	156	66	27.1		HC021	162	77	29.5
SX022	158	67	26.6		HC022	168	71	25.0
SX023	161	68	26.2		HC023	168	100	35.6
SX024	164	78	28.8		HC024	163	81	30.7
SX025	160	65	25.2		HC025	165	75	27.5
SX026	166	64	23.4					
SX027	154	66	27.6					
SX028	152	58	25.1					
SX029	154	88	37.1					
SX030	163	68	25.8					
SX031	152	65	27.9					
SX032	167	80	28.7					
SX033	162	72	27.2					
SX034	161	83	31.8					
SX035	161	94	36.1					
SX036	170	77	26.6					
SX037	162	68	26.0					
SX038	160	73	28.5					
SX039	167	71	25.4					
SX040	160	61	23.6					
SX041	166	75	27.0					
SX042	169	79	27.7					
SX043	161	68	26.2					
SX044	156	65	26.7					
SX045	158	73	29.4					
SX046	165	96	35.1					
SX047	157	79	32.0					
SX048	153	102	43.7					
SX049	160	88	34.4					
SX050	164	62	23.0					
SX051	157	68	27.7					
SX052	163	92	34.7					
SX053	159	80	31.6					
SX054	163	74	27.9					
SX055	152	82	35.3					
SX056	167	60	21.6					
Mean	**159**	**72**	**28.6**			**165**	**68**	**25.1**





Two-Sample T-Test: Body Mass Index

Group	n	Mean (kg/m^2)	Range	Standard Deviation	Standard Error of Mean
Syndrome X	56	28.58	20.4-43.7	4.61	0.62
Controls	25	25.13	18.9-35.6	3.84	0.77

Mean Difference (kg/m^2)	3.445	
95% Confidence interval for difference	lower	higher
	+1.469	+5.421
p value	0.001	

Two-Sample T-Test: Height

Group	n	Mean (cm)	Range	Standard Deviation	Standard Error of Mean
Syndrome X	56	159.43	147-170	5.15	0.69
Controls	25	164.92	152-173	4.72	0.94

Mean Difference (cm)	-5.49	
95% Confidence interval for difference	lower	higher
	-7.84	-3.15
p value	<0.001	

There is a statistically significant difference in the body mass index (BMI) of the women between the 2 groups. Women with syndrome x have a BMI which is higher by almost 3.5 kg/m^2 compared to healthy female controls. This is in part explained by the difference in height. Women in the Syndrome X group were over 5cm shorter on average than healthy controls.

3 : Anthropometric Data

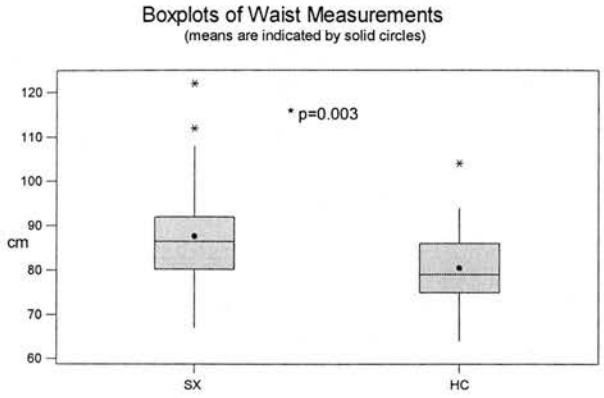
56 patients with Syndrome X listed with waist, hip, height and weight results at baseline along with 25 healthy controls.

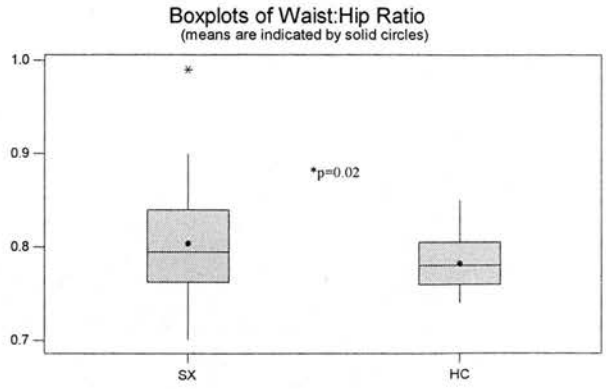
Syndrome X

Patient ID	Waist (cm)	Hip (cm)	W:H ratio
SX001	100	118	0.85
SX002	78	107	0.73
SX003	85	102	0.83
SX004	112	113	0.99
SX005	82	110	0.75
SX006	67	88	0.76
SX007	69	98	0.70
SX008	85	99	0.86
SX009	92	117	0.79
SX010	90	108	0.83
SX011	96	121	0.79
SX012	70	96	0.73
SX013	81	112	0.72
SX014	103	119	0.87
SX015	92	108	0.85
SX016	78	102	0.76
SX017	79	102	0.77
SX018	91	120	0.76
SX019	77	99	0.78
SX020	82	105	0.78
SX021	82	104	0.79
SX022	87	106	0.82
SX023	75	103	0.73
SX024	89	110	0.81
SX025	77	100	0.77
SX026	83	99	0.84
SX027	80	103	0.78
SX028	79	101	0.78
SX029	102	120	0.85
SX030	89	109	0.82
SX031	85	102	0.83
SX032	89	107	0.84
SX033	87	102	0.85
SX034	99	117	0.85
SX035	108	127	0.85
SX036	91	112	0.81
SX037	82	108	0.76
SX038	91	115	0.79
SX039	85	108	0.79
SX040	76	100	0.76
SX041	90	117	0.77
SX042	89	112	0.80
SX043	87	109	0.80
SX044	79	100	0.79
SX045	83	106	0.78
SX046	98	118	0.83
SX047	84	113	0.75
SX048	122	141	0.86
SX049	87	114	0.76
SX050	83	94	0.88
SX051	86	103	0.84
SX052	100	131	0.76
SX053	102	114	0.90
SX054	92	112	0.82
SX055	102	119	0.86
SX056	77	94	0.82
Mean	**87.6**	**108.8**	**0.80**

Healthy Controls

Patient ID	Waist (cm)	Hip (cm)	W:H ratio
HC001	85	101	0.85
HC002	80	108	0.74
HC003	78	104	0.75
HC004	83	108	0.77
HC005	83	99	0.84
HC006	85	109	0.78
HC007	79	102	0.78
HC008	90	112	0.81
HC009	78	101	0.78
HC010	74	95	0.78
HC011	75	97	0.77
HC012	87	112	0.78
HC013	79	100	0.79
HC014	72	93	0.77
HC015	65	87	0.75
HC016	75	94	0.80
HC017	77	101	0.76
HC018	76	101	0.75
HC019	74	97	0.76
HC020	64	86	0.74
HC021	91	110	0.83
HC022	76	100	0.76
HC023	104	128	0.81
HC024	94	119	0.79
HC025	90	110	0.82
Mean	**80.6**	**103.0**	**0.78**





Two-Sample T-Test: Waist

Group	n	Mean (cm)	Range	Standard Deviation	Standard Error of Mean
Syndrome X	56	87.6	67-122	10.6	1.4
Controls	25	80.6	52-100	8.94	1.8

Mean Difference (cm)	7.05	
95% Confidence interval for difference	lower	higher
	+2.46	+11.63
p value	**0.003**	

Two-Sample T-Test: W:H ratio

Group	n	Mean (cm)	Range	Standard Deviation	Standard Error of Mean
Syndrome X	56	0.804	0.70-0.99	0.051	0.007
Controls	25	0.782	0.74-0.85	0.031	0.006

Mean Difference	0.022	
95% Confidence interval for difference	lower	higher
	0.004	0.040
p value	**0.020**	

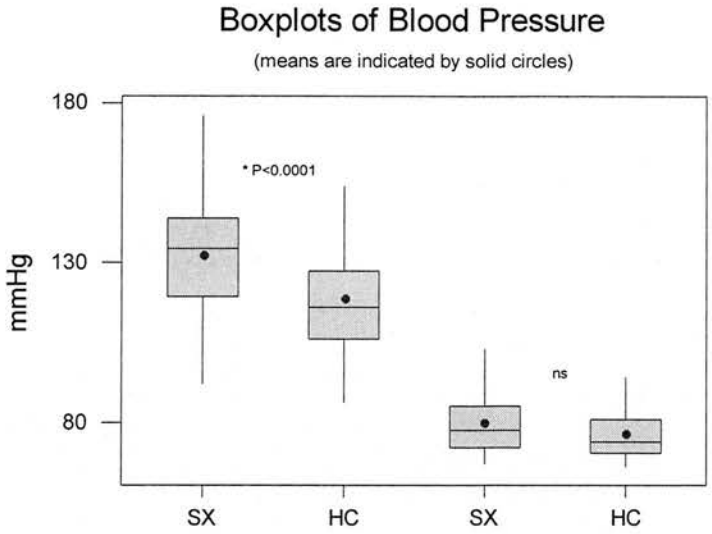
There is a statistical difference in waist measurement between the 2 groups and also in the waist:hip ratio but this is less significant than the waist measurement alone.

4: Blood Pressure

56 patients with Syndrome X listed with average systolic and diastolic blood pressure at baseline along with 25 healthy controls.

Syndrome X *Healthy Controls*

Patient ID	S BP (mmHg)	DBP (mmHg)		Control ID	SBP (mmHg)	DBP (mmHg)
SX001	135	86		HC001	127	82
SX002	99	67		HC002	110	72
SX003	126	75		HC003	112	66
SX004	139	67		HC004	133	87
SX005	111	73		HC005	107	68
SX006	118	74		HC006	107	70
SX007	128	79		HC007	125	79
SX008	132	68		HC008	148	92
SX009	156	85		HC009	121	81
SX010	151	87		HC010	109	71
SX011	135	97		HC011	116	74
SX012	134	75		HC012	105	73
SX013	157	93		HC013	116	75
SX014	146	78		HC014	121	76
SX015	144	86		HC015	114	67
SX016	121	71		HC016	125	75
SX017	176	103		HC017	130	74
SX018	136	82		HC018	128	86
SX019	143	76		HC019	100	71
SX020	119	73		HC020	136	71
SX021	127	85		HC021	154	94
SX022	136	77		HC022	99	69
SX023	147	81		HC023	118	81
SX024	148	87		HC024	100	68
SX025	133	74		HC025	102	74
SX026	149	85				
SX027	140	87				
SX028	160	85				
SX029	137	92				
SX030	110	67				
SX031	148	77				
SX032	108	69				
SX033	153	83				
SX034	129	82				
SX035	131	67				
SX036	123	76				
SX037	124	81				
SX038	125	70				
SX039	92	70				
SX040	152	85				
SX041	121	72				
SX042	144	87				
SX043	141	72				
SX044	119	75				
SX045	142	90				
SX046	138	91				
SX047	112	71				
SX048	144	98				
SX049	103	75				
SX050	111	73				
SX051	141	73				
SX052	133	82				
SX053	118	85				
SX054	146	91				
SX055	115	70				
SX056	108	69				
Average	**132**	**79**		**Average**	**119**	**76**



Two-Sample T-Test: Systolic Blood Pressure

Group	n	Mean (mmHg)	Range	Standard Deviation	Standard Error of Mean
Syndrome X	56	132.4	99-176	16.8	2.2
Controls	25	118.5	99-154	14.5	2.9

Mean Difference (mmHg)	13.87	
95% Confidence interval for difference	lower	higher
	+6.51	+21.24
p value	<0.001	

Two-Sample T-Test: Diastolic Blood Pressure

Group	n	Mean (mmHg)	Range	Standard Deviation	Standard Error of Mean
Syndrome X	56	79.45	67-103	8.84	1.2
Controls	25	75.84	67-94	7.65	1.5

Mean Difference (mmHg)	3.61	
95% Confidence interval for difference	lower	higher
	-0.27	7.48
p value	0.068	

There is a significant difference in the systolic blood pressure but not the diastolic blood pressure between the 2 groups. Women in the syndrome x group have a systolic blood pressure on average almost 15mmHg higher than the control subjects.

5 :

a) History of Chest Pain and Risk Factors

	Synd X	*Controls*
Median CCS* Index (+/- inter-quartile range)	1 (1)	-
Daily Frequency of Chest Pain	0.66	-
Daily Frequency of GTN use	0.45	-

* *Canadian Cardiovascular Society*

Family History of Angina/MI	37 (66%)	16 (64%)	ns
Family History of Type 2 Diabetes	22 (39%)	9 (36%)	ns
Gestational Diabetes	1 (2%)	1 (4%)	ns
Hypertension During Pregnancy	14 (25%)	2 (8%)	ns
Hysterectomy	16 (29%)	1 (4%)	* p=0.012
Peri / postmenopausal	34 (61%)	16 (64%)	ns
Smokers	8 (14%)	2 (8%)	ns
Ex-smoker (given up >10 years)	9 (16%)	6 (24%)	ns
Total with Smoking History	17 (30%)	8 (32%)	ns

Drugs	*Synd X Assessment*	*Synd X During study*	*Healthy Controls*	
Aspirin	41 (73%)	41 (73%)	0	p<0.001†
Beta-Blockers	16 (29%)	1 (2%)	0	ns†
Amlodipine	7 (13%)	1 (2%)	1 (4%)	ns†
Diltiazem	19 (34%)	3 (5%)	1 (4%)	ns†
Nitrates	14 (25%)	5 (9%)	0	ns†
Nicorandil	5 (9%)	3 (5%)	0	ns†
Statin	22 (39%)	22 (39%)	2 (8%)	p=0.004†
HRT	14 (25%)	14 (25%)	6 (24%)	ns†

† comparison between Syndrome X during study and Control groups

	Syndrome X (Assessment)
Number on at least one anti-anginal	39 (70%)
Number on at least two anti-anginals	14 (25%)

Number on at least three anti-anginals	4 (7%)

Chest Pain and Anti-anginals

Patients with cardiac 'Syndrome X' have much morbidity and this is reflected by data shown in the previous page. Amongst the 'Syndrome X' cohort, the average daily chest pain index was 0.66 indicating an average of 2 episodes of chest pain every 3 days. The use of GTN is not insignificant with an average of just over 3 administrations every week, as calculated from the daily index of 0.45.

This ongoing burden of symptoms is reinforced by the data describing the use of anti-anginals. Over 70% of women with Syndrome X were taking at least 1 anti-anginal at assessment with 25% taking at least 2 and 7% requiring 3 anti-anginal drugs. However, for the study duration most anti-anginals were stopped in the 'Syndrome X' group such that the rate of use of these drugs did not differ significantly with the control cohort.

The use of HMG-CoA Reductase inhibitors was significantly higher in the 'Syndrome X' group as compared with the control group. This difference is derived from the Chi-Squared test ($p=0.004$). Data on lipid profile is presented in the next section.

Past Medical and Family History

From the above table it is clear that the women in these 2 groups are similar in terms of the number with a family history of both ischaemic heart disease and type 2 diabetes. There are no significant differences in the smoking history between the groups with a similar percentage of current smokers and a similar percentage of previous smokers. These statistics were analysed by the 2x2 chi squared test.

b) Characteristics in terms of Menopause and Obstetric Data

	Syndrome X	Controls	
Peri/Postmenopausal*	34 (61%)	16 (64%)	ns
On HRT	14 (25%)	6 (24%)	ns
Oestrogen Deficient†	29 (52%)	9 (36%)	ns
Hysterectomy	16 (29%)	1 (4%)	* p=0.012

*amennorrhoeic for >12 months and FSH >30iU/L
† oestradiol <150 μmol/L

Hypertension during Pregnancy‡	14 (25%)	2 (8%)	ns
Gestational Diabetes‡	1 (2%)	1 (4%)	ns

‡ incidence reported by patient
Statistical p values obtained from the chi-squared test.

There is no statistically significant difference between the groups in terms of the numbers of women who are peri or postmenopausal. This state was confirmed by an oestradiol of less than 150micromol/L in conjunction with a raised FSH/LH (see section 10). There is no statistical difference in the number of women taking hormone-replacement therapy between the 2 groups.

There is, however, a significant difference in the number of women who have undergone hysterectomy in previous years between the 2 groups – 29% of the 'Syndrome X 'group compared with 4% of the controls. This finding has been documented by previous groups.

Women in the 'Syndrome X' group tended towards having problems with hypertension during pregnancy but the figure did not achieve statistical significance. No observable difference in the numbers with gestational diabetes was seen. These figures are based on self-reporting of gestational diabetes/hypertension, and as such may potentially lack accuracy.

6 : Insulin and Glucose

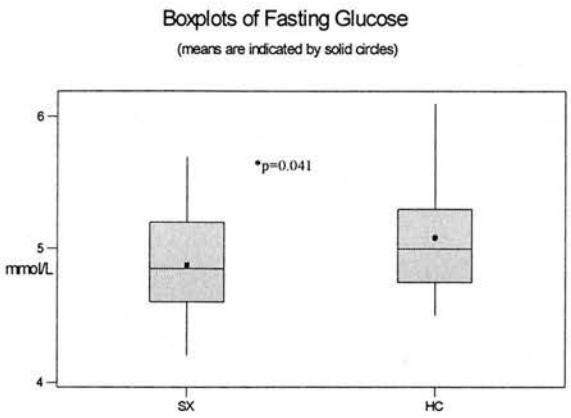
56 patients with Syndrome X listed with fasting insulin, glucose and non-esterified fatty acid results at baseline along with 25 healthy controls.

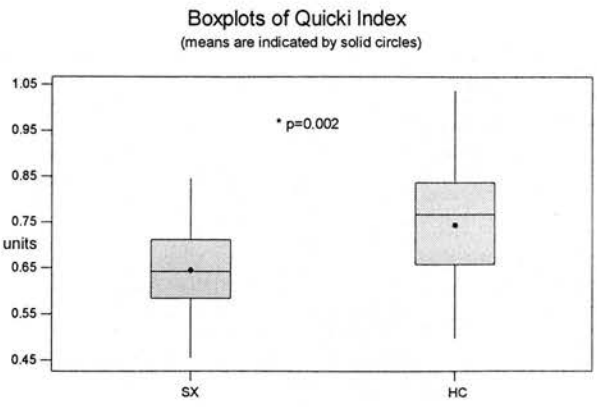
Syndrome X

Patient ID	Gluc (mmol/L)	Ins (mU/L)	Quicki	NEFA (mmol/L)
SX001	5.0	8.3	0.6180	0.55
SX002	4.4	4.3	0.7831	0.46
SX003	5.2	15.2	0.5269	0.77
SX004	4.7	33.6	0.4549	0.67
SX005	4.5	3.7	0.8187	0.80
SX006	4.7	5.6	0.7041	0.47
SX007	4.6	3.3	0.8465	0.45
SX008	4.4	5.0	0.7449	0.53
SX009	4.4	5.7	0.7146	0.84
SX010	4.4	4.3	0.7831	0.72
SX011	4.4	8.2	0.6422	0.70
SX012	5.3	5.6	0.6791	0.54
SX013	4.8	3.7	0.8004	0.89
SX014	4.6	5.5	0.7127	0.84
SX015	5.2	5.7	0.6794	0.36
SX016	4.5	7.0	0.6674	0.24
SX017	5.3	5.4	0.6865	0.66
SX018	4.2	4.5	0.7834	0.67
SX019	4.5	14.2	0.5539	0.80
SX020	5.4	7.0	0.6339	0.72
SX021	4.6	9.2	0.6148	0.58
SX022	4.7	9.4	0.6078	0.46
SX023	5.0	7.9	0.6263	0.55
SX024	4.3	7.6	0.6604	0.49
SX025	4.7	3.6	0.8141	0.57
SX026	4.7	4.2	0.7720	0.35
SX027	4.5	6.6	0.6790	0.42
SX028	5.0	11.7	0.5659	0.65
SX029	4.6	11.2	0.5841	0.82
SX030	4.9	7.1	0.6487	0.43
SX031	5.5	18.2	0.4999	0.59
SX032	4.9	8.0	0.6276	0.42
SX033	5.0	4.6	0.7344	0.42
SX034	5.5	13.9	0.5310	0.56
SX035	5.7	22.7	0.4735	0.51
SX036	5.0	7.7	0.6307	0.54
SX037	4.4	4.4	0.7771	0.43
SX038	5.0	6.9	0.6503	0.55
SX039	5.6	9.0	0.5874	0.52
SX040	5.6	6.5	0.6406	0.47
SX041	5.2	6.9	0.6431	0.40
SX042	4.8	14.7	0.5410	0.50
SX043	5.3	11.3	0.5626	0.62
SX044	5.3	7.9	0.6166	0.14
SX045	4.7	7.4	0.6488	0.34
SX046	5.0	26.6	0.4708	0.26
SX047	4.7	5.4	0.7120	0.41
SX048	5.5	7.3	0.6236	0.73
SX049	4.7	7.2	0.6538	0.51
SX050	5.1	8.2	0.6168	0.35
SX051	4.3	5.4	0.7321	0.37
SX052	4.9	9.7	0.5963	0.57
SX053	4.6	7.5	0.6503	0.53
SX054	5.1	18.0	0.5095	1.10
SX055	4.9	19.7	0.5039	0.51
SX056	5.5	13.8	0.5318	0.32
Mean	**4.9**	**9.2**	**0.650**	**0.55**

Healthy Controls

Control ID	Gluc (mmol/L)	Ins (mU/L)	Quicki	NEFA (mmol/L)
HC001	6.0	14.4	0.5164	0.42
HC002	4.8	3.2	0.8429	0.39
HC003	5.3	7.8	0.6187	0.54
HC004	4.9	2.2	0.9684	0.71
HC005	5.0	3.1	0.8401	0.48
HC006	5.1	2.7	0.8780	0.26
HC007	5.7	4.3	0.7198	0.51
HC008	5.1	4.5	0.7349	0.68
HC009	4.7	3.3	0.8399	0.44
HC010	5.1	1.8	1.0386	0.22
HC011	5.3	4.4	0.7311	0.38
HC012	4.5	3.5	0.8352	0.46
HC013	4.8	3.7	0.8004	0.44
HC014	6.1	3.3	0.7670	0.38
HC015	5.0	3.9	0.7752	0.15
HC016	5.3	5.2	0.6943	0.55
HC017	4.7	10.2	0.5950	1.08
HC018	5.3	3.7	0.7737	0.19
HC019	4.9	4.1	0.7675	0.53
HC020	5.5	3.1	0.8119	0.71
HC021	4.9	5.7	0.6915	0.47
HC022	4.7	7.7	0.6416	0.27
HC023	5.3	14.0	0.5346	0.49
HC024	4.6	22.2	0.4977	0.32
HC025	4.6	6.6	0.6746	0.38
Mean	**5.1**	**5.9**	**0.744**	**0.46**





Two-Sample T-Test: Fasting Plasma Glucose

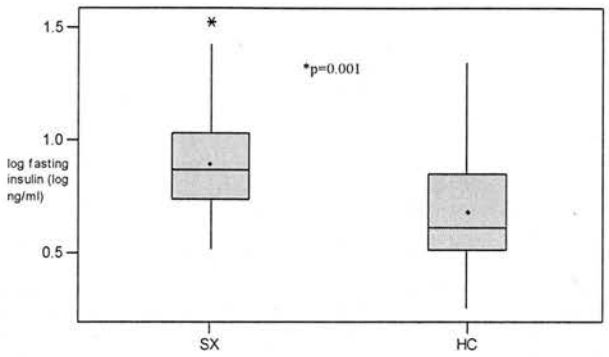
Group	n	Mean (mmol/L)	Range	Standard Deviation	Standard Error of Mean
Syndrome X	56	4.88	4.2-5.7	0.39	0.052
Controls	25	5.09	4.5-6.1	0.42	0.084

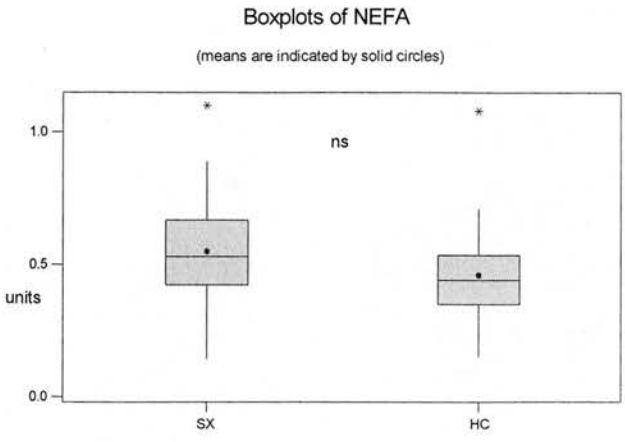
Mean Difference (mmol/L)	-0.208	
95% Confidence interval for difference	lower	higher
	-0.407	-0.009
p value	**0.041**	

Two-Sample T-Test: Quicki Index

Group	n	Mean (units)	Range	Standard Deviation	Standard Error of Mean
Syndrome X	56	0.6459	0.455-0.847	0.096	0.013
Controls	25	0.7440	0.498-1.039	0.132	0.026

Mean Difference (units)	-0.098	
95% Confidence interval for difference	lower	higher
	-0.157	-0.038
p value	**0.002**	





Two-Sample T-Test: log Fasting Insulin

Group	n	Mean (log mU/L)	Range (mU/L)	Standard Deviation	Standard Error of Mean
Syndrome X	56	0.896	3.3-33.6	0.228	0.031
Controls	25	0.684	1.8-22.2	0.265	0.053

Mean Difference (mU/L)	0.2122	
95% Confidence interval for difference	lower	higher
	+0.089	+0.336
p value	0.001	

Two-Sample T-Test: Non-esterified Fatty Acids

Group	n	Mean (mmHg)	Range	Standard Deviation	Standard Error of Mean
Syndrome X	56	0.548	0.14-1.10	0.179	0.024
Controls	25	0.458	0.15-1.08	0.197	0.039

Mean Difference (mmHg)	0.0897	
95% Confidence interval for difference	lower	higher
	-0.003	0.183
p value	0.058	

The fasting glucose is significantly lower in the Syndrome X group as compared to the healthy controls. This is likely to be a statistical effect of the relatively small group sizes. The quicki index is lower and the fasting insulin is higher in the Syndrome X group reflecting higher levels of insulin resistance, despite a lower fasting plasma glucose. There is, however, no significant difference in the fasting NEFAs between the groups.

7 : Lipids

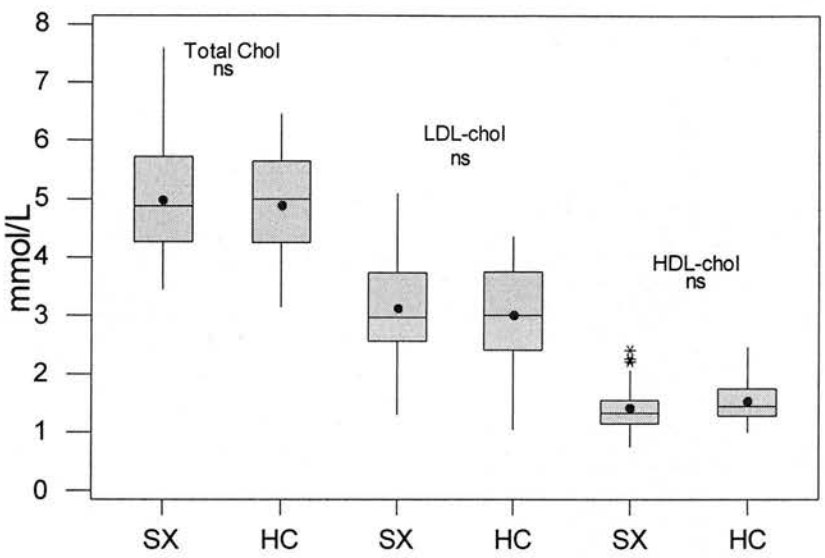
56 patients with syndrome x listed with fasting lipid results at baseline along with 25 healthy controls.

Syndrome X

Pat id	T Chol mmol/L	Recip Trig	VLDL mmol/L	LDL mmol/L	HDL mmol/L
SX001	5.45	0.8333	0.25	3.75	1.45
SX002	6.95	0.6250	0.4	5.1	1.45
SX003	6.5	0.5405	0.7	4.6	1.2
SX004	3.45	0.8000	0.3	2.1	1.05
SX005	5.45	0.7692	0.35	2.85	2.25
SX006	6.05	1.3333	0.35	3.85	1.85
SX007	4.45	0.9524	0.3	2.85	1.3
SX008	5.4	1.1111	0.35	3.25	1.8
SX009	5.6	0.9091	0.25	3.3	1.6
SX010	5.6	0.5263	0.35	3.5	1.75
SX011	5.75	0.6061	0.45	4.15	1.15
SX012	4.8	1.1111	0.4	2.9	1.5
SX013	4.75	0.7692	0.05	3.3	1.4
SX014	5.25	0.3448	1.15	3	1.1
SX015	6.25	0.8000	0.25	4.25	1.75
SX016	5.75	0.5128	0.7	4	1.05
SX017	5.8	0.7143	0.35	3.65	1.2
SX018	4.3	1.1765	0.25	2.5	1.55
SX019	5.4	0.4545	0.85	3.25	1.3
SX020	4.75	0.6897	0.25	3.2	1.3
SX021	4.3	1.0000	0.2	2.55	1.55
SX022	5.95	0.6452	0.45	3.8	1.7
SX023	5.15	1.5385	0.25	2.7	2.2
SX024	4.2	0.8333	0.2	2.8	1.2
SX025	6.55	1.3333	0.55	4.15	1.85
SX026	5.9	1.4286	0.3	4.15	1.45
SX027	4.15	0.9091	0.35	2.6	1.2
SX028	3.45	0.7143	0.3	1.35	1.8
SX029	6.3	0.7143	0.45	3.6	2.05
SX030	4.3	0.4651	0.8	2.7	0.8
SX031	4.55	1.1765	0.3	2.75	1.5
SX032	5.65	1.1765	0.5	3.95	1.2
SX033	5.8	0.6250	0.2	3.8	1.8
SX034	5.1	0.6250	0.65	3.4	1.05
SX035	4.25	0.3846	0.9	2.35	1
SX036	4.45	1.0526	0.25	2.7	1.5
SX037	5.45	1.0000	0.4	3.8	1.25
SX038	4.7	0.9524	0.35	3	1.35
SX039	3.5	0.5882	0.4	1.7	1.4
SX040	4.65	0.8000	0.35	2.95	1.35
SX041	3.9	0.8696	0.5	2.55	0.85
SX042	4.6	0.5882	0.4	3	1.2
SX043	5.2	0.3030	1.1	3.15	0.95
SX044	3.6	1.1111	0.15	2.3	1.15
SX045	3.65	0.8696	0.4	2.15	1.1
SX046	4.95	0.8696	0.45	3.7	0.8
SX047	3.9	1.2500	0.3	2.35	1.25
SX048	5.25	0.6452	0.4	2.45	2.4
SX049	4.2	1.0000	0.35	2.55	1.3
SX050	4.1	1.0000	0.3	2.65	1.15
SX051	4.55	0.9091	0.5	2.95	1.1
SX052	4.3	0.3704	0.7	2.25	1.35
SX053	6.2	0.8696	0.4	4.45	1.35
SX054	7.6	0.0565	5.55	1.3	0.75
SX055	3.7	0.7143	0.35	2.25	1.1
SX056	4.05	1.1765	0.45	2.2	1.4
Mean	**4.99**	**0.824**	**0.513**	**3.08**	**1.38**

Healthy Controls

Cont id	T Chol mmol/L	Recip Trig	VLDL mmol/L	LDL mmol/L	HDL mmol/L
HC001	6.45	0.2817	0.95	4.15	1.35
HC002	4.50	1.2500	0.50	2.40	1.60
HC003	4.15	0.6452	0.50	2.30	1.35
HC004	5.00	1.1765	0.35	3.25	1.40
HC005	5.80	1.1111	0.40	4.00	1.40
HC006	5.15	1.0000	0.45	3.00	1.70
HC007	4.80	1.4286	0.30	2.55	1.95
HC008	5.15	1.8182	0.10	2.60	2.45
HC009	4.10	1.6667	0.15	2.35	1.60
HC010	4.25	1.4286	0.20	2.40	1.65
HC011	3.75	1.8182	0.10	2.20	1.45
HC012	5.60	0.8696	0.60	3.85	1.15
HC013	4.45	0.8000	0.30	3.00	1.15
HC014	5.00	1.0000	0.15	3.05	1.80
HC015	5.70	1.2500	0.20	3.35	2.15
HC016	5.00	1.1111	0.35	3.25	1.40
HC017	5.05	1.8182	0.30	3.15	1.60
HC018	5.95	1.1765	0.40	3.65	1.90
HC019	5.80	1.0526	0.25	4.35	1.20
HC020	3.50	1.1111	0.25	1.70	1.55
HC021	6.15	0.7692	0.70	4.10	1.35
HC022	3.15	2.5000	0.15	1.05	1.95
HC023	5.50	0.6250	0.40	3.95	1.15
HC024	4.25	0.6452	0.60	2.65	1.00
HC025	4.80	0.3774	1.00	2.80	1.00
	4.92	**1.149**	**0.386**	**3.00**	**1.53**



Two-Sample T-Test: Total Cholesterol

Group	n	Mean (mmol/L)	Range	Standard Deviation	Standard Error of Mean
Syndrome X	56	4.996	3.45-7.6	0.955	0.13
Controls	25	4.920	3.15-6.45	0.845	0.17

Mean Difference (mmol/L)	0.076	
95% Confidence interval for difference	lower	higher
	-0.349	+0.501
p value	**0.720**	

Two-Sample T-Test: LDL-Cholesterol

Group	n	Mean (mmol/L)	Range	Standard Deviation	Standard Error of Mean
Syndrome X	56	3.079	1.30-5.10	0.800	0.11
Controls	25	3.004	1.05-4.35	0.816	0.16

Mean Difference (mmol/L)	0.075	
95% Confidence interval for difference	lower	higher
	-0.318	+0.467
p value	**0.704**	

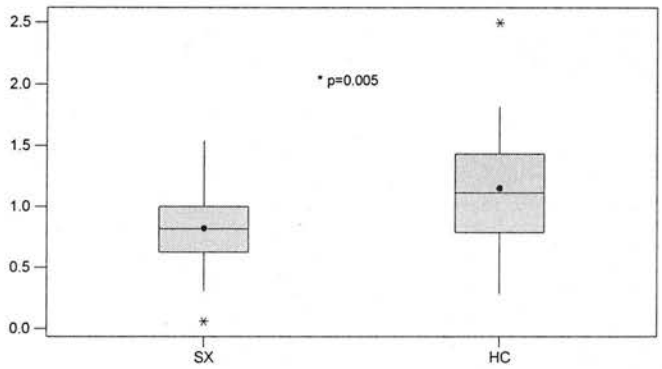
Two-Sample T-Test: HDL-Cholesterol

Group	n	Mean (mmol/L)	Range	Standard Deviation	Standard Error of Mean
Syndrome X	56	1.382	0.8-2.4	0.361	0.048
Controls	25	1.530	1.0-2.45	0.360	0.072

Mean Difference (mmol/L)	-0.148	
95% Confidence interval for difference	lower	higher
	-0.323	+0.027
p value	**0.095**	

Boxplots of Triglycerides (Reciprocal Transformation)

(means are indicated by solid circles)



Two-Sample T-Test: Reciprocal Triglycerides

Group	n	Mean (recip mmol/L)	Range	Standard Deviation	Standard Error of Mean
Syndrome X	56	0.824	0.30-1.25	0.301	0.040
Controls	25	1.149	0.28-2.5	0.506	0.10

Mean Difference (recip mmol/L)	-0.325	
95% Confidence interval for difference	lower	higher
	-0.547	-0.103
p value	**0.005**	

There is a significant difference in serum triglycerides between the two groups (women in the Syndrome X group have significantly higher triglycerides). No difference in serum total, LDL or HDL cholesterol is seen. This is despite more women in the syndrome x group taking statins (39% Vs 8%)

8 : Serum Endothelial Markers

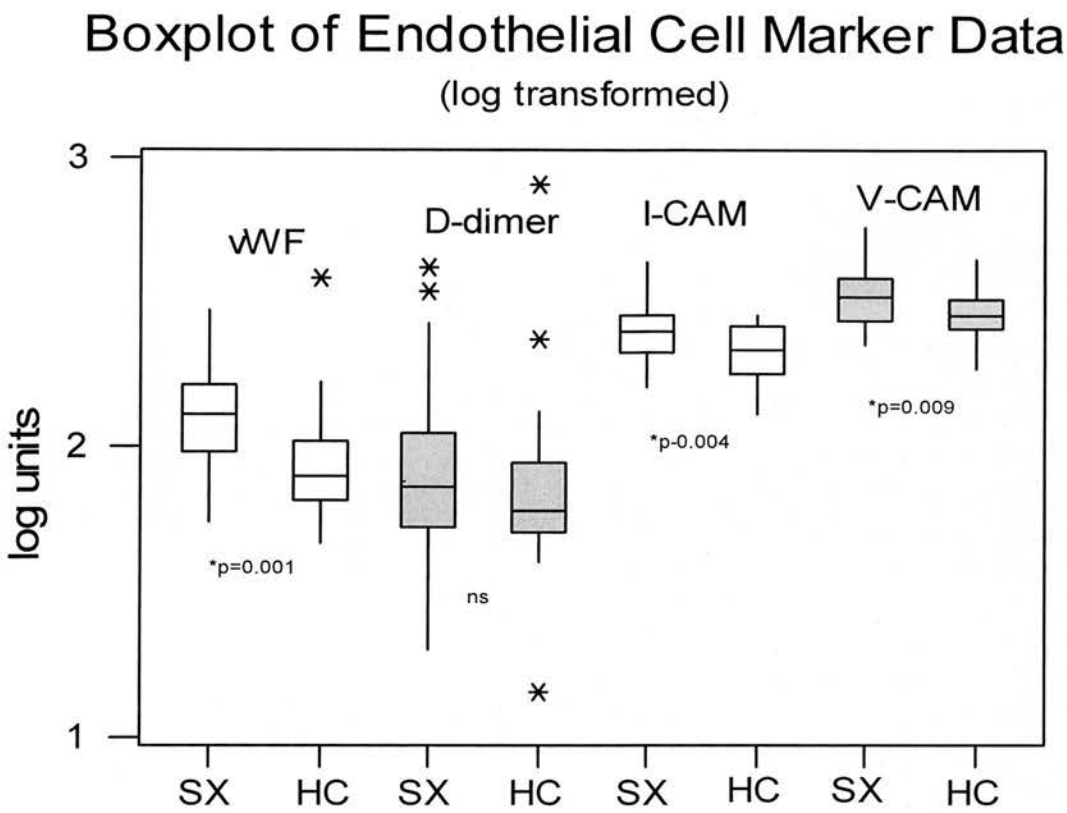
56 patients with syndrome x listed with fasting endothelial marker results at baseline along with 25 healthy controls.

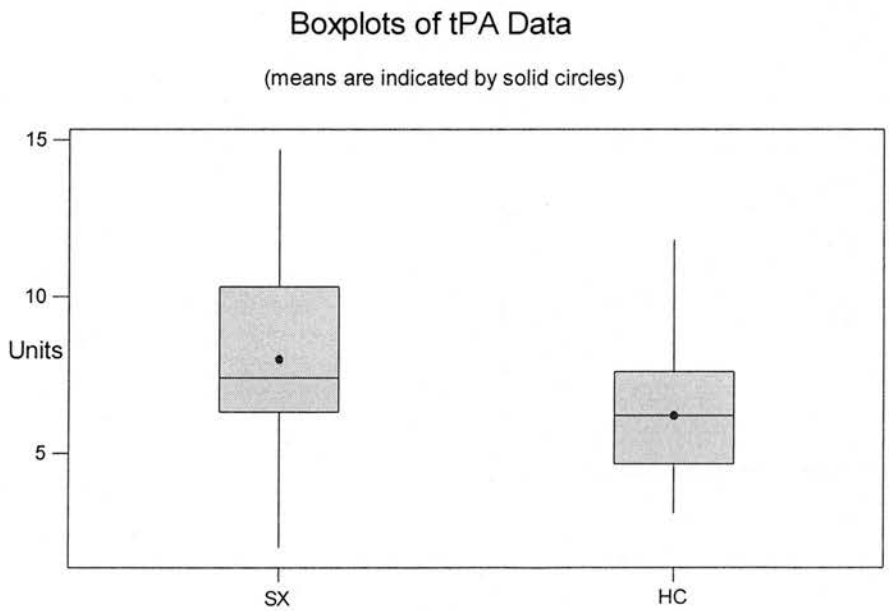
Syndrome X

Patient id	log vWF log %	tPA ng/ml	log D-d log units	log ICAM log ng/ml	log VCAM log ng/ml
SX001	2.11	9.6	1.785	2.360	2.474
SX002	2.12	6.7	1.580	2.256	2.362
SX003	2.39	9.2	1.826	2.452	2.489
SX004	2.11	11.3	1.806	2.462	2.596
SX005	2.14	4.3	1.929	2.400	2.370
SX006	1.81	5.0	1.792	2.603	2.406
SX007	2.32	5.9	1.826	2.454	2.584
SX008	2.23	7.4	2.290	2.507	2.492
SX009	2.01	5.3	1.672	2.477	2.660
SX010	2.12	2.0	1.672	2.269	2.393
SX011	2.22	11.6	1.672	2.347	2.415
SX012	2.19	6.7	1.380	2.335	2.435
SX013	2.07	6.9	2.049	2.383	2.377
SX014	2.23	7.7	1.833	2.467	2.461
SX015	2.17	10.8	1.869	2.391	2.526
SX016	1.98	6.7	1.924	2.369	2.547
SX017	2.23	12.1	2.625	2.454	2.598
SX018	2.21	6.5	2.013	2.452	2.769
SX019	2.09	10.5	1.580	2.643	2.481
SX020	2.17	4.0	1.924	2.418	2.432
SX021	2.09	3.8	1.919	2.343	2.648
SX022	2.08	8.0	1.301	2.373	2.558
SX023	2.22	6.7	1.869	2.426	2.660
SX024	1.96	14.7	2.241	2.453	2.547
SX025	2.08	4.9	1.748	2.304	2.446
SX026	2.13	6.2	1.681	2.208	2.485
SX027	1.94	7.0	2.025	2.358	2.547
SX028	2.20	7.0	1.531	2.247	2.574
SX029	2.11	6.5	2.100	2.414	2.535
SX030	2.23	13.1	1.792	2.559	2.580
SX031	2.29	10.5	2.037	2.419	2.594
SX032	2.03	10.0	1.653	2.329	2.468
SX033	1.76	4.3	2.004	2.314	2.477
SX034	2.15	9.9	1.924	2.608	2.542
SX035	2.27	13.2	2.079	2.496	2.632
SX036	2.15	6.5	2.346	2.253	2.541
SX037	1.93	3.8	1.740	2.416	2.449
SX038	1.98	7.9	2.068	2.345	2.591
SX039	1.86	7.4	1.491	2.425	2.446
SX040	1.82	6.3	1.771	2.306	2.515
SX041	2.25	10.0	1.748	2.293	2.509
SX042	2.00	9.9	2.288	2.307	2.382
SX043	2.15	12.8	1.935	2.414	2.413
SX044	2.04	6.7	1.806	2.327	2.573
SX045	1.98	9.6	2.217	2.294	2.379
SX046	1.81	10.7	1.863	2.296	2.409
SX047	2.14	3.8	1.505	2.491	2.689
SX048	2.23	12.1	2.212	2.358	2.512
SX049	1.83	6.4	2.041	2.363	2.597
SX050	2.00	11.2	2.548	2.280	2.431
SX051	2.26	7.4	2.428	2.642	2.770
SX052	1.82	7.5	1.924	2.442	2.637
SX053	2.08	5.5	2.137	2.560	2.566
SX054	1.74	8.0	1.716	2.469	2.612
SX055	2.48	10.4	1.708	2.424	2.526
SX056	2.27	6.9	1.924	2.471	2.615
Mean	**2.09**	**7.98**	**0.274**	**0.380**	**0.402**

Healthy Controls

Control id	log vWF log %	tPA ng/ml	log D-d log units	log ICAM log ng/ml	log VCAM log ng/ml
HC001	2.05	11.8	1.699	2.431	2.511
HC002	1.72	4.5	1.716	2.290	2.445
HC003	2.12	7.1	2.377	2.117	2.412
HC004	2.59	4.3	1.724	2.351	2.658
HC005	1.67	5.3	1.826	2.356	2.403
HC006	1.84	4.8	1.785	2.263	2.275
HC007	1.97	3.3	2.124	2.414	2.355
HC008	2.12	6.2	1.863	2.425	2.343
HC009	1.83	7.7	1.756	2.364	2.451
HC010	2.23	3.1	2.380	2.398	2.515
HC011	1.88	5.3	1.602	2.243	2.373
HC012	1.81	6.6	2.913	2.352	2.496
HC013	1.71	7.7	1.623	2.325	2.478
HC014	1.88	5.3	1.602	2.241	2.425
HC015	1.93	7.2	1.959	2.455	2.525
HC016	1.90	7.6	1.771	2.331	2.423
HC017	2.00	5.4	1.778	2.160	2.504
HC018	1.97	7.0	1.672	2.239	2.444
HC019	2.04	6.6	1.940	2.247	2.460
HC020	1.90	3.9	1.146	2.302	2.571
HC021	1.77	8.8	1.833	2.456	2.435
HC022	1.88	3.2	1.869	2.344	2.593
HC023	1.97	8.3	1.892	2.453	2.501
HC024	1.83	6.2	2.045	2.285	2.514
HC025	1.79	7.6	1.785	2.461	2.533
	1.94	**6.19**	**0.265**	**0.368**	**0.392**


Boxplot of Endothelial Cell Marker Data
(log transformed)
log units
3
2
1
vWF
D-dimer
I-CAM
V-CAM
*p=0.001
ns
*p-0.004
*p=0.009
SX
HC
SX
HC
SX
HC
SX
HC


Boxplots of tPA Data
(means are indicated by solid circles)
Units
15
10
5
SX
HC

Two-Sample T-Test: log vWF

Group	n	Mean (log %)	Range	Standard Deviation	Standard Error of Mean
Syndrome X	56	2.09	1.74-2.48	0.162	0.022
Controls	25	1.94	1.67-2.59	0.193	0.039

Mean Difference (log %)	0.1583	
95% Confidence interval for difference	lower	higher
	+0.0689	+0.2477
p value	**0.001**	

Two-Sample T-Test: log d-dimer

Group	n	Mean (log units)	Range	Standard Deviation	Standard Error of Mean
Syndrome X	56	1.900	1.30-2.63	0.273	0.036
Controls	25	1.867	1.15-2.91	0.329	0.066

Mean Difference (log units)	0.0323	
95% Confidence interval for difference	lower	higher
	-0.1200	0.1846
p value	**0.670**	

Two-Sample T-Test : log I-CAM

Group	n	Mean (log ng/ml)	Range	Standard Deviation	Standard Error of Mean
Syndrome X	56	2.402	2.25-2.64	0.101	0.013
Controls	25	2.332	2.12-2.46	0.094	0.019

Mean Difference (log ng/ml)	0.070	
95% Confidence interval for difference	lower	higher
	+0.0236	+0.116
p value	**0.004**	

Two-Sample T-Test: log V-CAM

Group	n	Mean (log ng/ml)	Range	Standard Deviation	Standard Error of Mean
Syndrome X	56	2.524	2.36-2.77	0.098	0.013
Controls	25	2.466	2.28-2.66	0.084	0.017

Mean Difference (log ng/ml)	0.0579	
95% Confidence interval for difference	lower	higher
	+0.0154	+0.1005
p value	**0.009**	

Two-Sample T-Test: tPA

Group	n	Mean (ng/ml)	Range	Standard Deviation	Standard Error of Mean
Syndrome X	56	7.98	2.0-14.7	2.84	0.38
Controls	25	6.19	3.1-11.8	2.02	0.40

Mean Difference (ng/ml)	1.787	
95% Confidence interval for difference	lower	higher
	+0.681	+2.892
p value	**0.002**	

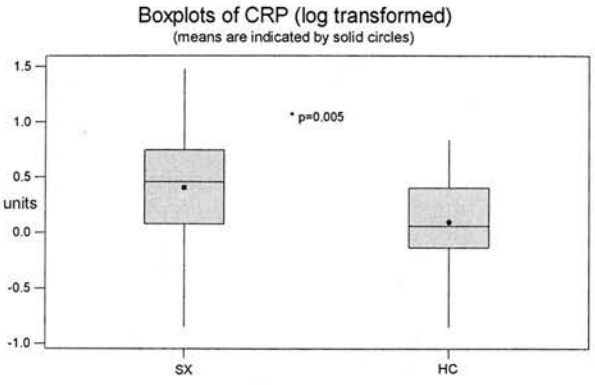
There is a significant difference in the endothelial cell factors – tPA, vWF, ICAM and VCAM between the 2 groups. These factors are all higher in the Syndrome X group. However no significant difference is seen in the D-dimers between the groups.

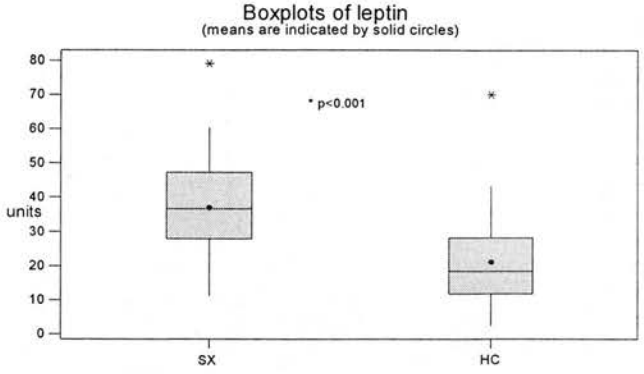
9 : C-reactive Protein and Leptin

56 patients with syndrome x listed with CRP and leptin results at baseline along with 25 healthy controls.

Syndrome X *Healthy Controls*

Patient ID	log CRP (log mg/L)	Leptin ng/L		Control ID	log CRP (log mg/L)	Leptin ng/L
SX001	-0.4815	58.4		HC001	0.5587	29.3
SX002	-0.3010	28.7		HC002	0.0607	12.0
SX003	0.4698	37.9		HC003	0.8420	19.9
SX004	1.1290	60.2		HC004	-0.1675	23.9
SX005	0.7543	28.8		HC005	0.0414	11.3
SX006	-0.2007	11.8		HC006	0.0374	18.5
SX007	0.4031	19.3		HC007	0.8370	19.9
SX008	0.3075	27.8		HC008	0.5441	36.5
SX009	0.3054	35.4		HC009	-0.8539	14.1
SX010	0.8938	40.8		HC010	0.1790	8.3
SX011	0.4378	49.9		HC011	-0.0177	18.8
SX012	0.0719	24.2		HC012	0.3784	27.2
SX013	1.0017	38.4		HC013	-0.0915	14.6
SX014	1.0073	38.0		HC014	-0.5376	17.1
SX015	-0.2757	33.0		HC015	-0.5229	8.8
SX016	0.1673	23.4		HC016	-0.0269	7.9
SX017	-0.0362	23.7		HC017	0.3385	13.1
SX018	0.7649	44.8		HC018	0.1430	16.6
SX019	0.4472	24.8		HC019	0.4298	11.6
SX020	0.7332	37.0		HC020	-0.4089	2.4
SX021	0.5038	44.9		HC021	0.5428	33.6
SX022	0.0334	34.0		HC022	-0.2218	20.5
SX023	-0.3010	42.0		HC023	-0.1024	70.2
SX024	0.6785	44.3		HC024	0.2148	43.4
SX025	0.1875	31.9		HC025	0.3222	31.4
SX026	-0.8539	15.3				
SX027	0.3692	30.7				
SX028	0.6637	41.3				
SX029	0.6085	36.4				
SX030	0.7497	16.9				
SX031	0.4393	57.7				
SX032	0.8351	47.7				
SX033	0.4518	29.7				
SX034	0.2148	41.6				
SX035	0.5416	79.2				
SX036	-0.5850	36.9				
SX037	-0.0969	33.2				
SX038	0.0899	21.3				
SX039	0.6474	29.4				
SX040	0.2788	11.2				
SX041	-0.2518	38.2				
SX042	0.6284	21.9				
SX043	0.6415	51.0				
SX044	0.7505	46.4				
SX045	0.6149	28.5				
SX046	-0.0555	58.0				
SX047	0.6821	50.9				
SX048	0.9101	49.8				
SX049	0.3784	52.5				
SX050	0.0374	48.6				
SX051	1.4783	26.8				
SX052	0.9557	60.6				
SX053	0.5763	36.2				
SX054	0.9020	32.6				
SX055	0.9096	52.2				
SX056	0.4713	11.2				
Mean	**0.405**	**37.09**			**0.101**	**21.24**





Two-Sample T-Test: log C-reaactive Protein

Group	n	Mean (log ng/ml)	Range	Standard Deviation	Standard Error of Mean
Syndrome X	56	0.405	-0.85-1.48	0.466	0.062
Controls	25	0.101	-0.85-0.84	0.422	0.084

Mean Difference (log ng/ml)	0.304	
95% Confidence interval for difference	lower	higher
	+0.094	+0.515
p value	**0.005**	

Two-Sample T-Test: Leptin

Group	n	Mean (ng/ml)	Range	Standard Deviation	Standard Error of Mean
Syndrome X	56	37.1	11.2-79.2	14.1	1.9
Controls	25	21.2	2.4-70.2	14.2	2.8

Mean Difference (ng/ml)	15.86	
95% Confidence interval for difference	lower	higher
	+9.00	+22.72
p value	**<0.001**	

There is a statistically significant difference in CRP and leptin between both groups. Both parameters are significantly higher among the women in the Syndrome X group.

10 : Oestrogens

56 patients with syndrome x listed with sex hormone profile result, hormone replace therapy (HRT) usage and oestrogen status at baseline along with 25 healthy controls.

Syndrome X

Patient ID	FSH	LH	Oest	HRT	Oes Status*
SX001	96.0	72.0	<50	0	0
SX002	2.1	1.4	1070	1	1
SX003	46.0	25.0	<50	0	0
SX004	5.6	8.6	360	0	1
SX005	57.0	55.0	120	0	0
SX006	12.0	9.5	630	0	1
SX007	110.0	56.0	<50	0	0
SX008	100.0	55.0	<50	0	0
SX009	65.8	34.7	<50	0	0
SX010	5.8	4.4	530	1	1
SX011	17.0	27.2	328	0	1
SX012	118.5	53.8	<50	0	0
SX013	2.9	3.0	433	1	1
SX014	0.5	0.5	<50	1	0
SX015	57.8	23.4	<50	0	0
SX016	81.2	67.4	<50	0	0
SX017	65.3	30.2	<50	0	0
SX018	23.3	30.3	580	0	1
SX019	67.6	42.2	198	1	1
SX020	11.8	15.5	367	1	1
SX021	3.60	6.80	307	0	1
SX022	43.40	19.70	<50	0	0
SX023	85.80	30.40	<50	0	0
SX024	2.60	3.00	521	0	1
SX025	54.10	26.90	<50	0	0
SX026	10.00	9.20	201	0	1
SX027	67.50	26.80	<50	0	0
SX028	5.40	10.20	734	0	1
SX029	68.30	43.00	<50	0	0
SX030	59.7	107.6	<50	0	0
SX031	64.3	28.8	<50	0	0
SX032	52.6	26.9	<50	0	0
SX033	15.7	9.5	469	1	1
SX034	34.0	24.8	<50	0	0
SX035	74.3	38.0	<50	0	0
SX036	80.6	37.5	<50	0	0
SX037	7.9	7.8	641	0	1
SX038	10.0	7.4	123	1	0
SX039	45.3	41.0	434	1	1
SX040	90.7	17.9	<50	0	0
SX041	69.6	32.0	<50	0	0
SX042	17.2	16.7	563	0	1
SX043	53.5	31.4	<50	0	0
SX044	58.1	35.1	<50	0	0
SX045	8.3	4.6	176	0	1
SX046	7.8	5.6	154	0	1
SX047	20.0	30.0	373	0	1
SX048	9.3	14.1	304	1	1
SX049	3.4	3.8	441	1	1
SX050	11.8	8.3	296	1	1
SX051	85.6	48.8	<50	0	0
SX052	36.1	30.3	472	1	1
SX053	18.5	9.7	314	0	1
SX054	8.3	12.5	626	1	1
SX055	5.8	15.4	603	0	1
SX056	64.1	57.6	106	0	0

Healthy Controls

Control ID	FSH	LH	Oest	HRT	Oes Status*
HC001	4.6	6.6	671	1	1
HC002	24.5	6.9	<50	0	0
HC003	0.5	0.5	625	1	1
HC004	80.2	70.5	261	0	1
HC005	5.1	2.4	292	0	1
HC006	20.8	11.3	<50	0	0
HC007	33.1	19.1	249	1	1
HC008	71.9	19.0	<50	0	0
HC009	42.5	31.9	416	0	1
HC010	8.7	10.6	319	1	1
HC011	8.4	3.8	117	0	0
HC012	5.2	3.6	347	0	1
HC013	5.5	0.9	239	0	1
HC014	45.6	37.3	132	0	0
HC015	90.1	47.5	<50	0	0
HC016	7.5	6.2	494	0	1
HC017	79.8	33.0	<50	0	0
HC018	68.2	38.9	<50	0	0
HC019	63.2	19.1	156	1	1
HC020	0.9	0.5	607	1	1
HC021	97.8	40.9	<50	0	0
HC022	4.9	1.8	202	0	1
HC023	4.0	1.9	241	0	1
HC024	1.1	0.5	507	0	1
HC025	3.9	7.2	839	0	1

'Oestrogen status' – see explanatory note in paragraph below.

	Syndrome X	Controls	
Peri/Postmenopausal	34 (61%)	16 (64%)	ns
On HRT	14 (25%)	6 (24%)	ns
Oestrogen Deficient	29 (52%)	9 (36%)	ns

Statistical p values obtained from the chi-squared test.

An oestrogen-deficient state was defined as <150micromol/L of oestradiol irrespective of the gonadotrophins or HRT status. An 'oestrogen status' of 0 denotes an oestrogen deficient state whereas an 'oestrogen status of 1 denotes a non oestrogen deficient state. There was no difference between the 2 groups. This has important implications for the next section, as impaired vasomotor function has been documented in postmenopausal oestrogen-deficient women.

Correcting for Age and BMI

There were significant differences between the 2 groups in terms of age and body mass index. Potentially, these could drive some of the differences between the other variables presented in this chapter. Therefore, correction is required for age and body mass index. One way of achieving this is by a technique termed regression analysis. The other method would be to exclude women from the groups such that the BMI and age of the 2 groups does match. Data from both these methods are presented.

1. Regression Analysis

Regression analysis is a statistical technique in whereby differences between the variables are sought not only in relation to whether they belong to the 'Syndrome X' or healthy control group, but also in relation to their age and BMI. The results of this regression analysis are presented in the table below with variables corrected for age, BMI then both age and BMI together. The goal of regression analysis is to obtain estimates of an unknown parameters Beta_1, ..., Beta_K which indicate how a change in one of the independent variables (ie age/BMI) affects the values taken by the dependent variable.

Results indicate that significant differences remain only between the groups for:

- Triglycerides
- Von Willebrand factor
- I-CAM
- Fasting glucose
- Quicki index of insulin sensitivity
- Fasting insulin
- Leptin

Loss of significance occurs for :

- Systolic blood pressure
- Tissue plasminogen activator
- V-CAM
- Waist
- Waist : Hip ratio
- C-reactive protein

Adjustment for age and BMI results in some variables gaining significance for difference between the groups:

- HDL-cholesterol

The unadjusted and adjusted results for the 'Syndrome X' patients and the controls are summarised in tables 4.1 and 4.2.

Table 4.1: p values for unpaired t test for variables compared between groups and the effect on the p value of regression analysis

Variable	Difference between groups (2 sample t test)	Corrected for age	Corrected for BMI	Corrected for age and BMI (regression analysis)
Sys Blood Pressure	***p<0.001*** *	***p=0.012*** *	***p=0.004*** *	p=0.133 ns reg coeff = 6.086 (SE 4.005) T = 1.52
Recip Triglycerides	***p=0.005*** *	***P<0.001*** *	***P=0.01*** *	***p=0.014*** * reg coeff = -0.2530 (SE 0.1007) T = -2.51
HDL-Cholesterol	p=0.095 ns	***p=0.014*** *	p=0.177 ns	***p=0.025*** * reg coeff = -0.21827 (SE 0.09577) T = -2.28
tPA	***p=0.002*** *	***p=0.014*** *	p=0.062 ns	p=0.2 ns reg coeff = 0.8935 (SE 0.6919) T = 1.29
log vWF	**p=0.001** *	**p=0.003** *	**p=0.001** *	**p= 0.015** * reg coeff = 0.02538 (SE 0.00973) T = 2.61
log I-CAM	***p=0.004*** *	***p=0.003*** *	***p=0.019*** *	***p=0.018*** * reg coeff = 0.0119 (SE 0.00494) T = 2.40
log V-CAM	***p=0.009*** *	***p=0.017*** *	***p=0.049****	p=0.083 ns reg coeff = 0.00782 (SE 0.00445) T = 1.76
Fasting glucose	***p=0.041*** *	***p=0.006*** *	p=0.106 ns	***p=0.02*** * reg coeff = -0.2540 (SE 0.1071) T = -2.37
log fasting insulin	***p=0.001*** *	***p<0.001****	***p=0.013****	***p=0.016**** reg coeff = 0.155 (SE 0.0626) T = 2.47
quicki index	***p=0.002*** *	***p<0.001*** *	***p=0.007*** *	***p=0.016*** * reg coeff = -0.0708 (SE 0.0289) T = -2.45
waist	***p=0.003*** *	***p=0.001*** *	p=0.979 ns	p=0.887 ns reg coeff = 0.184 (SE 1.293) T = 0.14
waist:hip ratio	***p=0.02*** *	***p=0.017*** *	p=0.519 ns	p=0.349 ns reg coeff = 0.01092 (SE 0.0116) T = 0.94
log CRP	***p=0.005*** *	***p=0.004*** *	p=0.115 ns	p=0.129 ns reg coeff = 0.1796 (SE 0.1171) T = 1.53
Leptin	***p<0.001*** *	***p<0.001*** *	***p=0.003*** *	***p=0.007*** * reg coeff = 7.608 (SE 2.754) T = 2.76

Table 4.2: Summary of results – differences between Syndrome X and Control Groups (mean with standard deviation in parenthesis)

	Syndrome X n=56	Controls n=25	p value	p value - BMI/age adjusted
Indices of insulin resistance				
fasting plasma glucose (mmol/L)	4.9 (0.39)	5.1 (0.42)	**0.041**	**0.02**
log fasting insulin* (mu/L)	7.87 (1.69)	4.83 (1.84)	**0.001**	**0.016**
Quicki index †	0.65 (0.096)	0.74 (0.132)	**0.002**	**0.016**
Blood Pressure				
Systolic blood pressure (mmHg)	132.4 (16.8)	118.5 (14.5)	**<0.001**	0.133
Diastolic blood pressure (mmHg)	79.5 (8.8)	75.8 (7.7)	0.068	0.547
Lipids				
Total cholesterol (mmol/L)	5.00 (0.96)	4.92 (0.85)	0.720	0.750
HDL-cholesterol (mmol/L)	1.38 (0.36)	1.53 (0.36)	0.095	**0.025**
Triglyceride* (mmol/L)	1.21(3.3)	0.87 (2.0)	**0.005**	**0.014**
Endothelial/inflammatory markers				
Von Willebrand Factor* (%)	123.0 (1.45)	87.1 (1.56)	**0.001**	**0.015**
tPA (ng/ml)	7.91 (2.9)	6.13 (2.0)	**0.002**	0.2
ICAM-1* (ng/ml)	252.3 (1.26)	214.8 (1.24)	**0.004**	**0.018**
VCAM* (ng/ml)	334.2 (1.25)	292.4 (1.21)	**0.009**	0.083
C-reactive protein* (mg/L)	2.54 (2.9)	1.26 (2.6)	**0.005**	0.129
Adiposity measures				
Waist (cm)	87.6 (10.6)	80.6 (8.9)	**0.003**	0.887
Waist:Hip ratio	0.80 (0.05)	0.78 (0.03)	**0.02**	0.349
Serum leptin (ng/ml)	37.1 (14.2)	21.2 (14.2)	**<0.001**	**0.007**

*Geometric mean and standard deviation. †Quicki index = (1/[(log insulin) + (log glucose)]

Analysis of Regression Analysis

Correcting for the differences in age and body mass index shows that significant differences persist only for specific variables. These include

- **Insulin and Glucose**

  Lower Quicki index of insulin sensitivity and higher fasting insulin for the Syndrome X group, suggesting the Syndrome X group is more insulin resistant. The lower fasting plasma glucose is difficult to explain in the light of this increased insulin resistance compared to the controls, and is likely to be a statistical consequence of relatively small sample sizes.

- **Lipid Profile**

  Less favourable profile for the Syndrome X group with higher triglycerides and lower HDL-cholesterol. This is despite significant number of the 'Syndrome X' group being on statin therapy.

- **Serum Endothelial Cell Markers**

  Higher levels of vWF and I-CAM in the Syndrome X group compared with control subjects. This suggests a potential difference in the function of endothelial cells between the 2 groups.

- **Leptin**

  Leptin is a serum marker associated with adiposity. The difference in leptin between the groups remains very significant even after adjustment for the differences in BMI and may hint that its effect is more wide ranging than just reflecting obesity levels. The difference between the groups in waist:hip ratio are rendered insignificant by BMI adjustment as would be expected. However, the difference in CRP is also removed by BMI adjustment and this suggests that inflammatory levels may be related to adiposity.

2. Excluding Group Members

Eliminating the age differences between the groups can also be achieved by excluding specific subjects from the analysis. The 2 groups are matched for age if the 4 oldest members of the Syndrome X cohort are excluded along with the youngest control group member. This involves excluding :

Syndrome X Group	Control Group
MR1911	HM0804
JC1306	
ET1101	
CG1405	

This generates the baseline demographics illustrated below in table 4.3

Table 4.3. ***Demographics of the patients with cardiac Syndrome X and healthy control group. Mean +/- 95% confidence interval of mean or n (%).***

	Syndrome X n=52	Healthy Controls n=24	P value
Age*	55.6 +/- 2.07	52.0 +/- 3.04	NS
Body Mass Index* (kg/m^2)	28.58 +/- 1.24	25.1 +/- 1.58	0.001
Fasting Glucose* (mmol/L)	4.9 +/- 0.1	5.1 +/- 0.2	0.022
Smokers	8 (15%)	1 (4%)	NS
Gynaecological Factors			
Hysterectomy	15 (29%)	1 (4%)	0.014
Peri/Postmenopausal	30 (58%)	16 (67%)	NS
On HRT	14 (27%)	6 (25%)	NS
Hypertension in Pregnancy	14 (27%)	1 (4%)	0.021
Medication			
Statin therapy	22 (42%)	2 (8%)	0.003

Excluding these members on the basis of their age ensures that the 2 groups are marched according to ages (see table 2). However, there does remain a significant difference between the BMI of the 2 groups. Many more subjects would require exclusion to make BMI comparable, and this would be at major expense of statistical power. Therefore, adjustment for BMI is still required by regression analysis to remove the direct effect of this variable on others measured.

The other variables are compared by an unpaired t test in a similar manner as earlier in this chapter. The results are not fully presented by means of boxplots as before but are merely summarised in table 4.4 below.

As can be seen, very similar findings are presented.

Significant differences are seen between the groups as before in :

- **Insulin and Glucose**

  Fasting insulin and quicki index of insulin resistance. The differences in fasting insulin and quicki index remain highly significant even after adjustment for body mass index, suggesting that the Syndrome X group are insulin resistant compared to the healthy controls. The lower fasting plasma glucose in the 'Syndrome X' group remains difficult to explain.

- **Blood Pressure**

  Systolic blood pressure between the groups is significantly different once the data is analysed in this manner. This difference remains significant after adjustment for BMI.

- **Lipid Profile**

  Differences in triglycerides and HDL-cholesterol are seen between the 2 groups with the Syndrome X cohort having an unfavourable profile compared to the healthy controls. Total and LDL-cholesterol are not significantly different between the groups. The differences between HDL-cholesterol and triglycerides persist after adjustment for BMI. Again the differences in the numbers of women taking statin therapy is different, with significantly more women in the 'Syndrome X' group taking a statin during the trial.

- **Serum Endothelial Cell Markers**

  Higher levels of vWF, I-CAM, V-CAM and t-PA were seen in the Syndrome X group compared with control subjects. With adjustment for BMI, significance was lost in the V-CAM and t-PA differences leaving significance only in the vWF and I-CAM differences, as seen with the prior method of analysis. This again suggests a difference in the function of endothelial cells between the 2 groups.

- **Leptin**

  The difference in leptin between the groups again remains very significant even after adjustment for the differences in BMI and may hint that its effect is more wide ranging than just influencing obesity levels. The CRP difference is lost as before once adjustment for BMI is made.

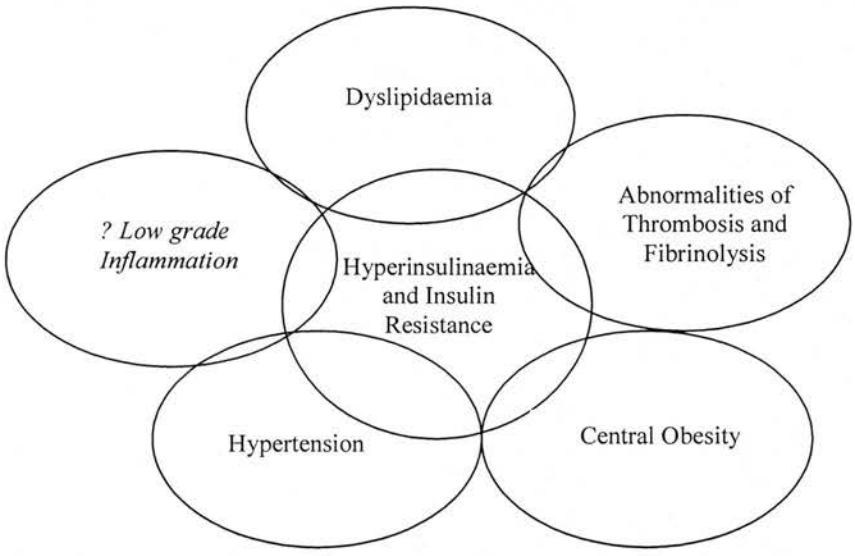
Table 4.4. ***Classical and novel risk factor parameters in cases and controls***

	Syndrome X n=52	Controls n=24	p value	p value - BMI adj.
Indices of insulin resistance				
log fasting insulin* (mu/L)	7.86 (1.70)	4.77 (1.86)	**0.001**	**0.016**
Quicki index †	0.65 (0.096)	0.75 (0.134)	**0.001**	**0.009**
Blood Pressure				
Systolic blood pressure (mmHg)	131.6 (17.1)	119.2 (14.4)	**0.002**	**0.017**
Diastolic blood pressure (mmHg)	79.6 (9.0)	75.9 (7.8)	0.078	0.547
Lipids				
Total cholesterol (mmol/L)	4.97 (0.97)	4.93 (0.86)	0.839	0.838
HDL-cholesterol (mmol/L)	1.35 (0.35)	1.55 (0.35)	**0.023**	**0.042**
Triglycerides* (mmol/L)	1.37 (1.7)	0.93 (1.6)	**0.002**	**0.018**
Endothelial/inflammatory markers				
Von Willebrand Factor* (%)	121.3 (1.46)	87.5 (1.56)	**0.004**	**0.005**
tPA (ng/ml)	7.91 (2.9)	6.13 (2.0)	**0.003**	0.082
ICAM-1* (ng/ml)	251.2 (1.3)	212.3 (1.2)	**0.003**	**0.021**
VCAM* (ng/ml)	331.3 (1.3)	290.3 (1.2)	**0.012**	0.067
C-reactive protein* (mg/L)	2.71 (2.9)	1.24 (2.7)	**0.003**	0.071
Adiposity measures				
Waist (cm)	87.9 (10.9)	80.2 (8.9)	**0.002**	0.855
Serum leptin (ng/ml)	36.9 (14.3)	20.8 (14.3)	**<0.001**	**0.005**

*Geometric mean and standard deviation. †Quicki index = (1/[(log insulin) + (log glucose)]

Correlations Between Indices of Insulin Resistance and Other Features of the Metabolic Syndrome

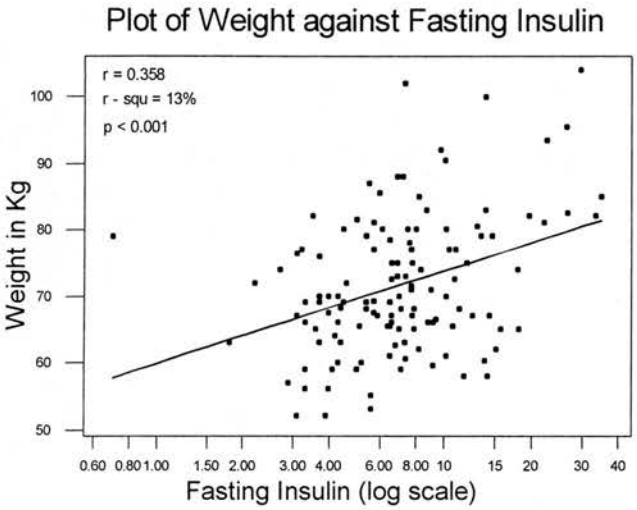
Resistance to the metabolic effects of insulin is thought to be the central factor in the metabolic syndrome, which was discussed in chapter 1. Briefly, it is a inter-related cluster of metabolic derangements which are known to pre-dispose to cardiovascular disease. This is represented schematically below.

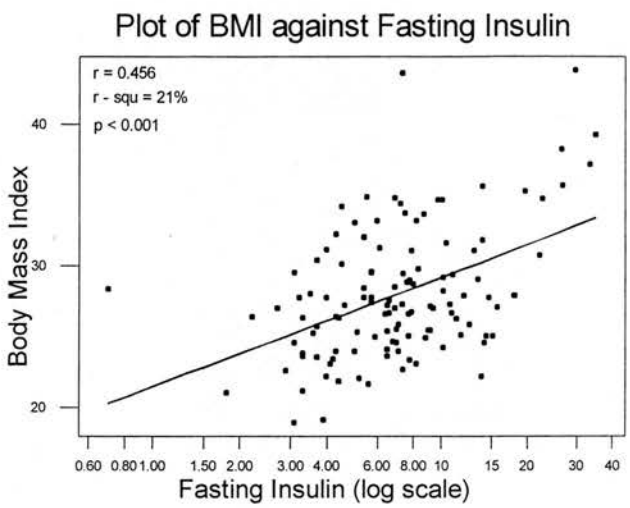


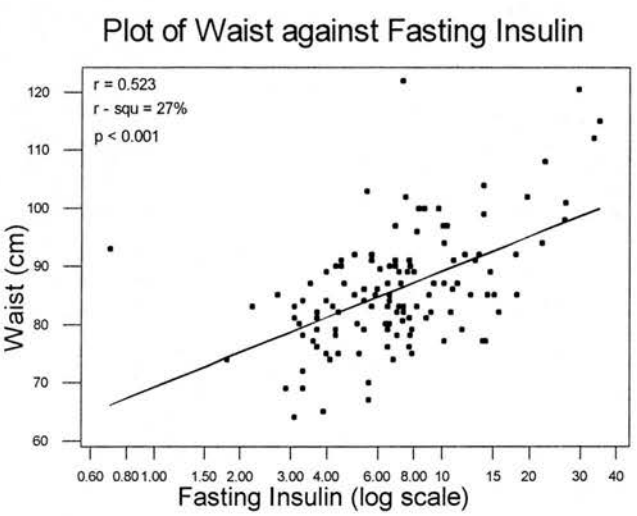
As discussed already in this chapter, the population of 56 women with cardiac 'Syndrome X' enrolled into the MIRS Study exhibited many features of the metabolic syndrome as compared with the 25 healthy controls. The following section sets out to describe the correlations seen between indices of insulin resistance (log transformed fasting insulin was found to be the best) and the other metabolic variables measured. Most of these relationships are well-described.

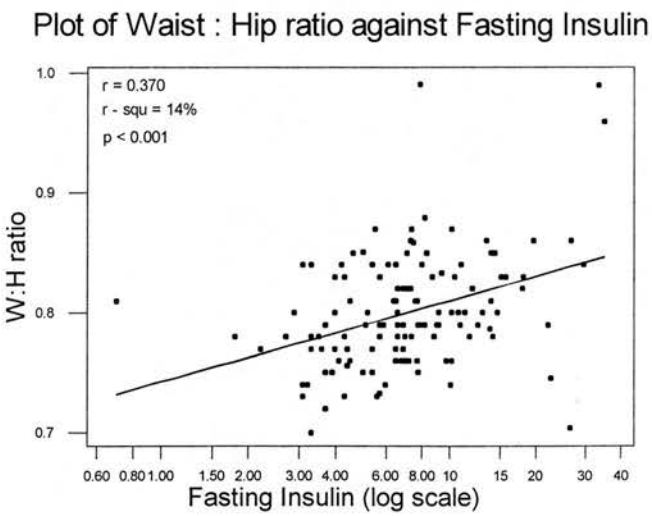
The correlations were made using data that was collected at baseline and after treatment with either placebo or metformin in the 'Syndrome X 'group. The data on the healthy controls were also included to give a total of 127 data points. Using all the available data ought to provide information on a more complete spectrum of insulin sensitivity.

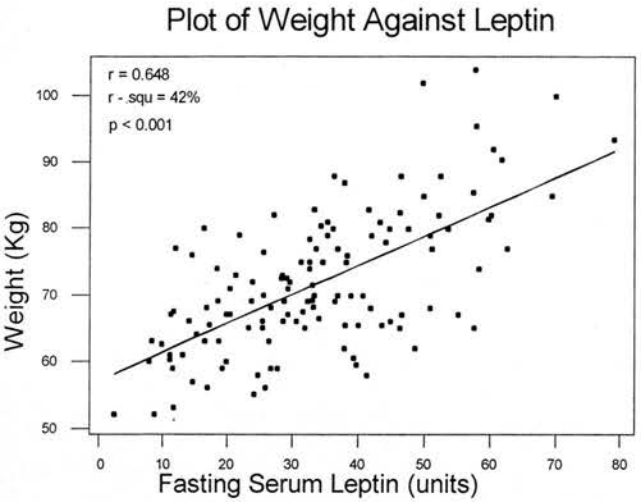
1 : *Anthropometric Variables*

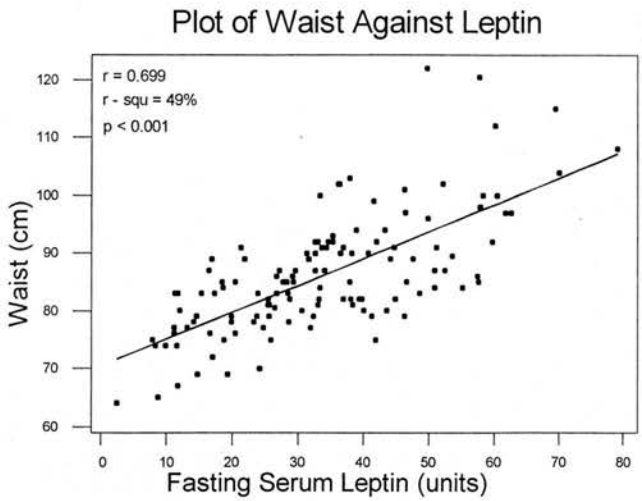


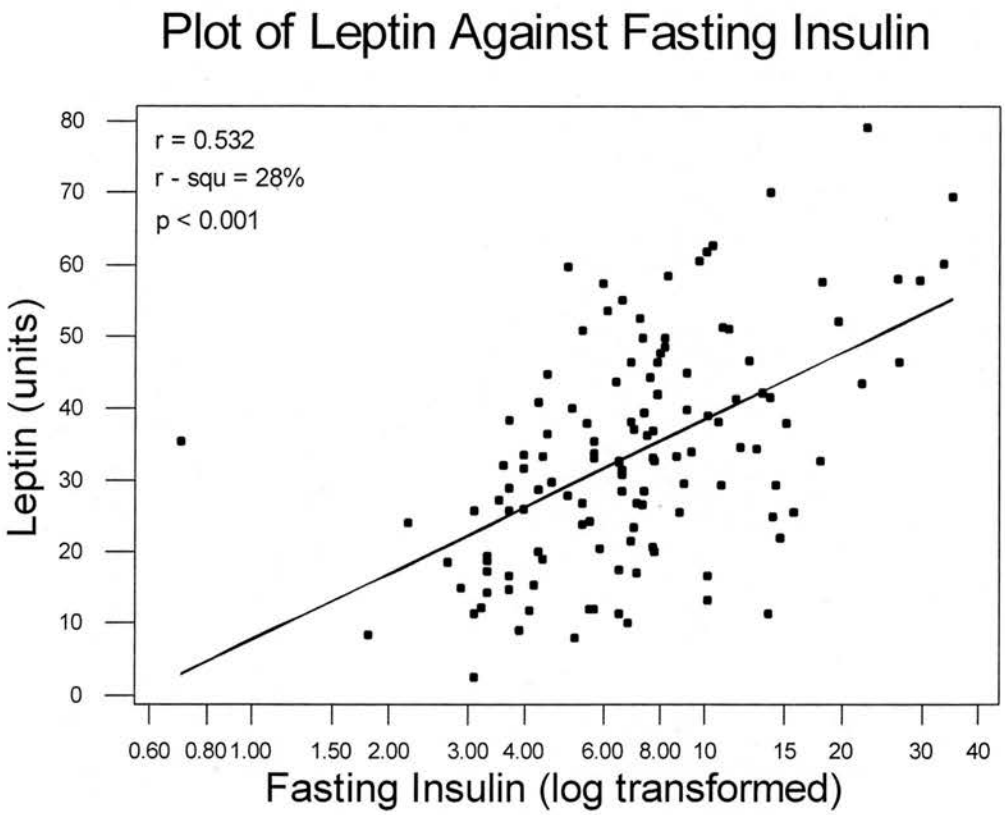






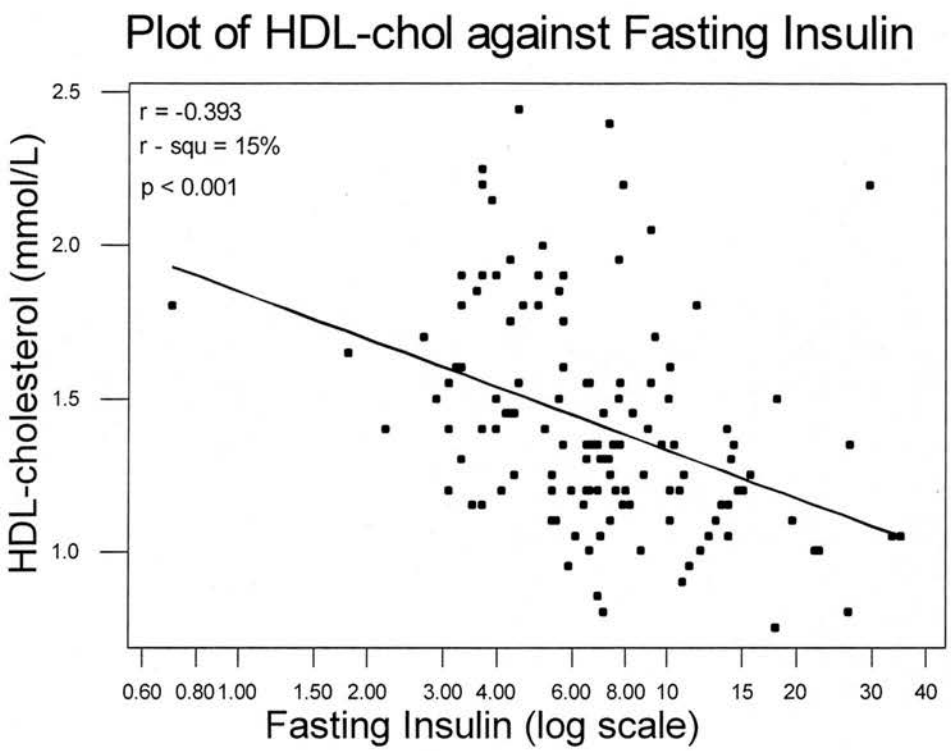


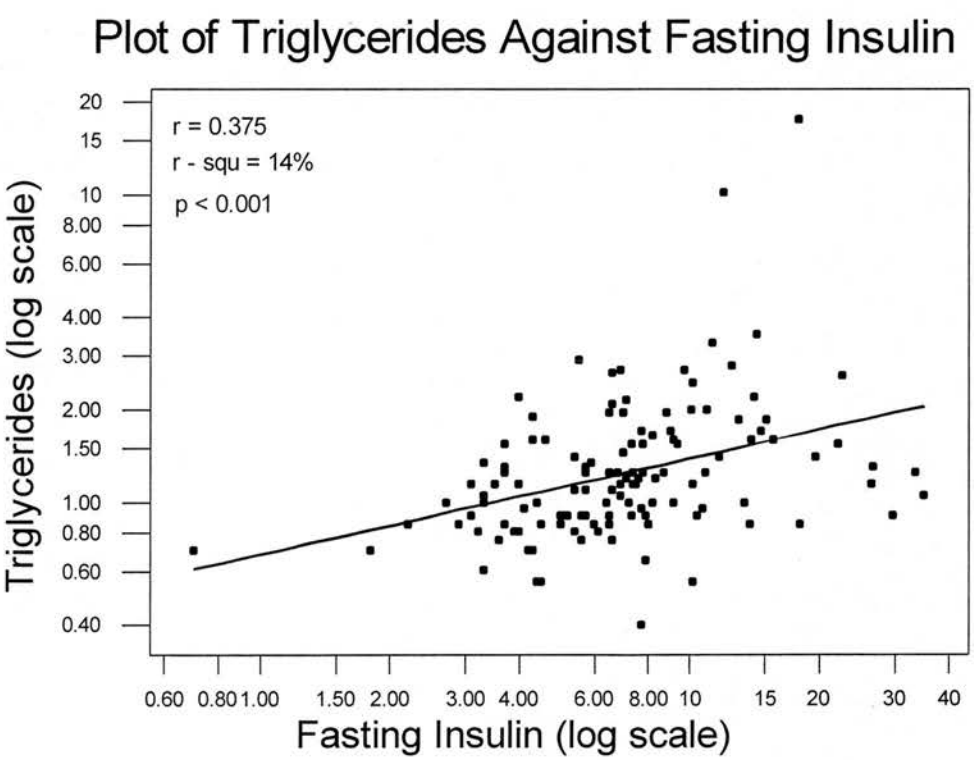




All of the anthropometric variables exhibit a significant relationship to insulin sensitivity. This tends to suggest that insulin resistance is related not only to body mass (total fat mass) but also waist diameter and, therefore, central adiposity. Serum leptin (thought to reflect fat mass) is strongly correlated to both total weight and waist diameter and, therefore, as expected has a robust association itself with insulin sensitivity.

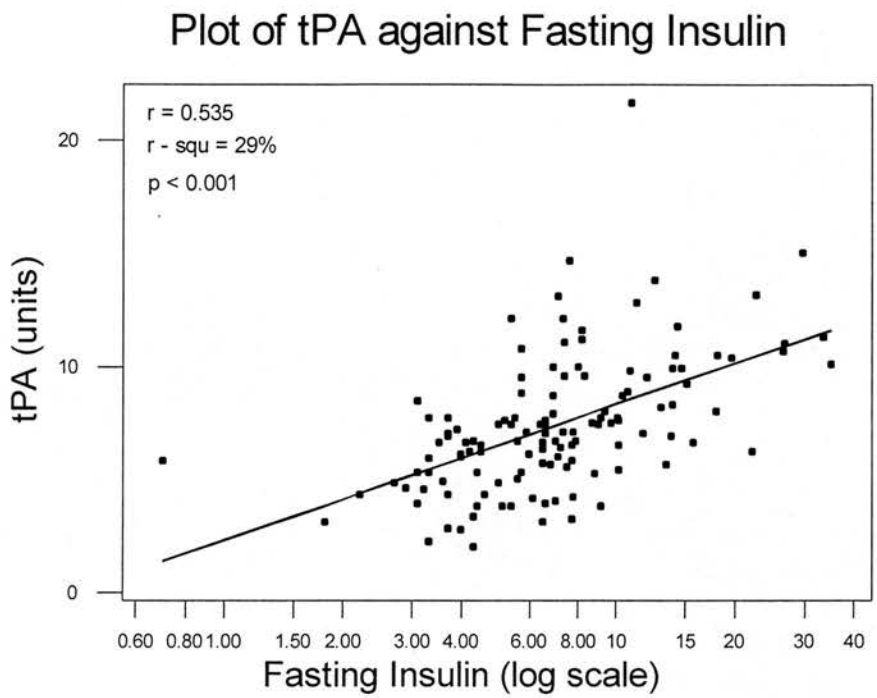
2 : *Lipid Profile*

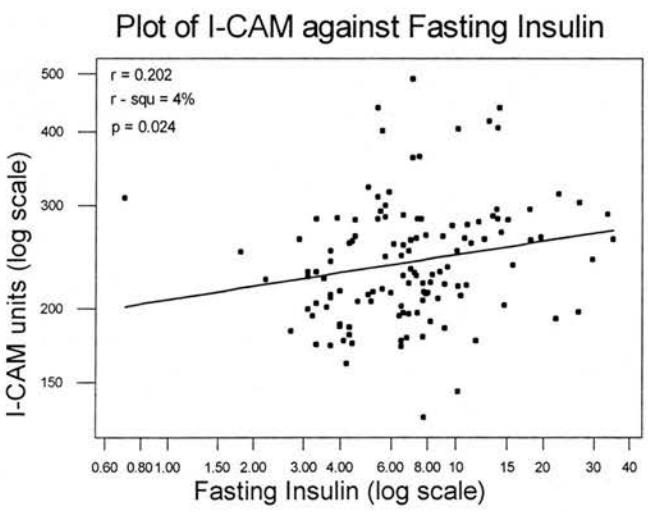


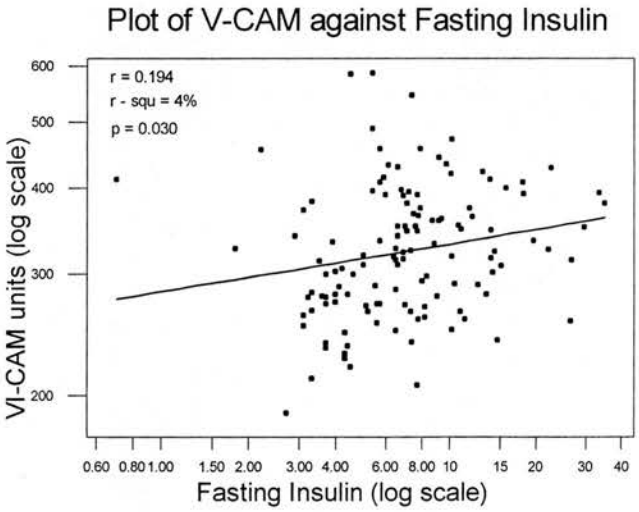


Significant correlations exist between both triglycerides and HDL-cholesterol and this index of insulin resistance. No relationship was observed for total cholesterol nor LDL-cholesterol.

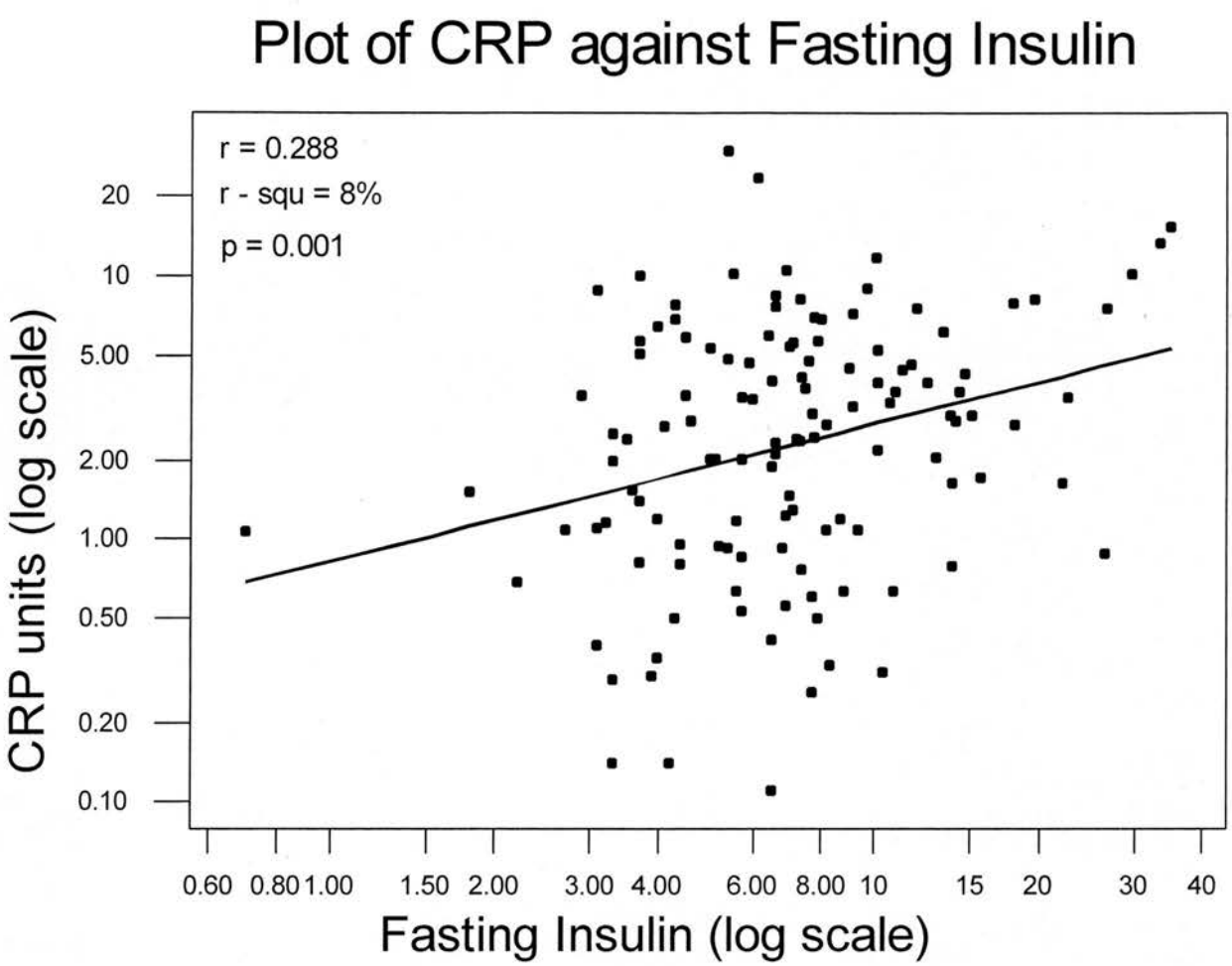
3 : *Serum Endothelial Markers*







There is a relatively strong correlation between tPA and insulin sensitivity and a much weaker association for the cellular adhesion molecules. No correlation was seen for D-dimers nor von Willebrand factor.



A fairly weak correlation exists between this index of insulin sensitivity and sensitive CRP. Many now regard inflammation as part of the metabolic syndrome and this relationship would tend to support this position.

Summary

Subjects within the cardiac 'Syndrome X' group exhibit a variety of metabolic differences compared to the cohort of healthy controls. Chance sampling resulted in the healthy controls being approximately 5 years younger than the 'Syndrome X' group. The body mass index of the 'Syndrome X' group was slightly but significantly greater than controls. Much of this effect was due to the average height of the control subjects being over 5cm greater than that of the subjects in the 'Syndrome X' cohort. This, in itself, is difficult to explain other than as a result of chance. However, the higher body mass index points to a greater proportion of body fat in the 'Syndrome X' group, also reflected by the higher leptin levels in this group.

The significant differences in fasting insulin and quicki index between the groups indicates higher levels of insulin resistance in the 'Syndrome X 'cohort, even when adjusted for the differences in BMI between the groups. This insulin resistance is sub-clinical and one would not expect any difference in fasting glucose between the groups (elevated fasting glucose was actually used as an exclusion criterion for the study). However, although fasting glucose for all subjects was 'normal', the mean fasting glucose was statistically lower in the subjects with 'Syndrome X'. This result is difficult to explain metabolically, and again is likely a chance finding given the relatively small sample sizes studied. Higher levels of insulin resistance would be expected in relation to the higher fat mass within the 'Syndrome X' group and indeed a modest correlation was seen between body mass index and fasting insulin (r^2 = 21%). My data on higher fasting insulin levels concur with considerable previous data in subjects with cardiac syndrome X (1-8).

The 'Syndrome X' cohort also show other features of the metabolic syndrome which go along with the insulin resistance. These include lipid perturbances namely relative hypertriglyceridaemia and lower levels of HDL-cholesterol. Higher systolic blood pressure was seen in the 'Syndrome X' when analysed such that subjects were excluded to facilitate age-matching between the groups. Higher CRP levels were also

seen in the 'Syndrome X' group, suggesting an inflammatory response, but these differences lost significance when BMI was taken into account.
Serum markers of endothelial dysfunction were elevated in the 'Syndrome X' group. These include von Willebrand factor and I-CAM but the differences in t-PA and V-CAM lost significance when corrected for age and BMI.

These data demonstrate that despite glucose concentrations within normal ranges, women with cardiac 'Syndrome X' have biochemical indices reflecting relative insulin resistance. They also demonstrate BMI-independent elevations in a number of novel parameters linked to insulin resistance and endothelial function namely leptin, vWF and ICAM-1, which have been shown to predict incident CHD. CRP was also higher in absolute terms but BMI adjustment reduced the difference in accordance with considerable evidence for an effect of adiposity on inflammatory status

These data are important and in some respects unique in that I have now extended the spectrum of risk factor abnormalities in women with Cardiac Syndrome X to include vWF, ICAM-1, leptin and potentially t-PA and CRP. The elevation in leptin is of particular interest since a recent report has linked leptin to vascular dysfunction independently of other pathways (9). These observations are relevant since many of these parameters are recent additions to the spectrum of perturbances linked to 'metabolic' syndrome X, and thus, in turn, to insulin resistance.

Correlations with insulin sensitivity were seen in several metabolic variables known to be associated with the metabolic syndrome. Although blood pressure did not exhibit correlation, associations of various strength with fasting insulin were seen with lipids, inflammatory markers, anthropometric data reflecting fat mass and central adiposity and some serum markers of endothelial function. Most of these correlations were fairly modest with relatively small correlation co-efficients. This reflects the fact that overall, insulin resistance per se probably plays a minor role in influencing these variables independently and that many other contributory factors are at work.

Interestingly, I noted that 27% of the cases (vs 4% of controls) reported problems with hypertension during pregnancy. This finding suggests that impaired peripheral microvascular function is associated with hypertensive complications during pregnancy. Recent data from our group and others have confirmed that women with a history of pre-eclampsia have impaired microvascular function by the techniques of flow-mediated dilatation (10) and laser Doppler imaging (11).

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CHAPTER 5

Assessing Peripheral Microvascular Response

Assessing Vascular Function

Vascular function has been assessed in various different groups using several different techniques in recent years. Impaired vasomotor function has been demonstrated in patients with hypertension (1), diabetes (2), sub-clinical insulin resistance (3) and coronary artery disease (4). Several techniques with varying degrees of invasiveness have been employed and some of these are listed in table 5.1 below.

Table 5.1:
Various methods of measuring vascular function listed with important characteristics

Method	Location	Measurement	Vessel Size	Invasive
Quantitative Coronary Angiography	coronary	Coronary artery diameter	Macro	Invasive: intra-coronary catheters
Coronary Fractional flow Reserve	coronary	Intra-coronary pressure	Macro	Invasive: intra- coronary catheters
Coronary thermodilution	coronary	Intra-coronary temperature	Macro/micro	Invasive: intra- coronary catheters
Intra-coronary Doppler	coronary	Intra-coronary flow velocity	Macro/micro	Invasive: intra- coronary catheters
Venous occlusion plethysmography	peripheral	Forearm plethysmography	Macro/micro	Invasive: peripheral arterial cannulation
Peripheral arterial ultrasound	peripheral	Arterial diameter	Macro	Non-invasive if used with reactive hyperaemia but may involve arterial or venous cannulation
Serum Markers	systemic	Quantitative values derived from serum assays	Micro/macro	Venous sampling only
Laser Doppler fluximetry	peripheral	Change in reflected Doppler wavelength	Micro	Non-invasive when drug delivery by iontophoresis
Laser Doppler imaging	peripheral	Change in reflected Doppler wavelength	Micro	Non-invasive when drug delivery by iontophoresis

Techniques listed above measure changes in vascular properties in relation to specific provocations which can be either endothelium-dependent or endothelium-independent. Examples of typical provocations include:

- Acetylcholine — normally produces endothelium-dependent vasodilation and subsequent increase in vascular flow.
- Nitrate — normally produces endothelium-independent vasodilation and subsequent increase in vascular flow.
- Adenosine — induces coronary vasodilatation in an endothelium-dependent and independent way.
- Dipyridamole — induces coronary vasodilatation in an endothelium dependent way.
- Cold Pressor Test — normally produces sympathetic-driven coronary vasodilatation.
- Reactive Hyperaemia — normally produces endothelium-dependent vasodilation after transient vascular occlusion.

The methods listed in the table above measure vascular function in different vascular beds as well as in vessels of varying calibre, from conduit arteries to capillary microcirculation. Many groups have discriminated between endothelium-dependent and independent mechanisms using different vasoactive drugs which act through different pathways. Nitrate donors such as sodium nitroprusside (SNP) can affect vascular smooth muscle vasodilation directly, whereas drugs such as acetylcholine (ACh) rely on an intact endothelial layer to couple their effects to the production of endothelial-derived vasodilators including endogenous nitric oxide (NO).

Vasomotor Dysfunction in Microvascular Angina

It has been postulated that reduced vasodilator reserve due to microvascular dysfunction is responsible for the generation of myocardial ischaemia in some patients with anginal chest pain and normal coronary arteries. This has been dealt with in more detail in chapter 2. Briefly, coronary vasomotor abnormalities have been documented in subsets of patients with cardiac 'Syndrome X' in response to pacing and intra-coronary drugs using direct coronary ultrasound, thermodilution, calculated coronary resistance and great cardiac vein catheterisation and flow measurements (5-9). Additionally, there is evidence of more widespread vascular dysfunction, with abnormalities being demonstrated in the forearm cutaneous vascular beds in response to relative ischaemia. Modalities used include plethysmography and brachial artery ultrasound (10-12).

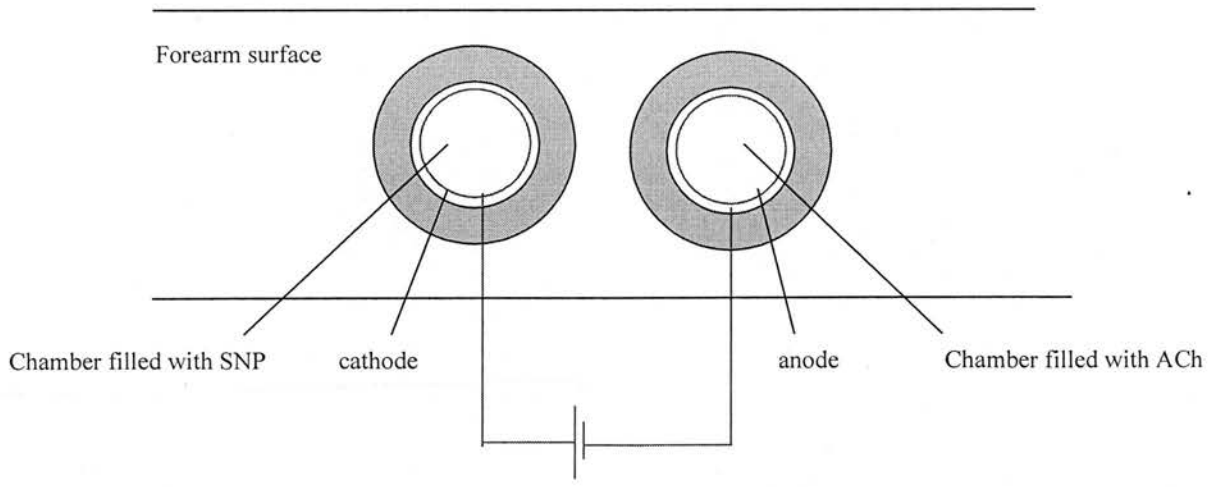
There are data that demonstrate correlations between the behaviour of coronary and peripheral (brachial) arteries in patients undergoing coronary angiography. Anderson et al looked at the coronary vasomotor response to intra-coronary acetyl choline (ACh) and found that those subjects who exhibited a coronary vasoconstrictor response, had attenuated brachial artery flow-mediated vasodilatation, as assessed by high resolution brachial artery ultrasound (13).

Measurement of Vascular Function using Laser Doppler Imaging

The measures of cutaneous vascular function performed as part of the MIRS study in both women with cardiac 'Syndrome X' and healthy controls were done using this non-invasive technique. Essentially, the imager detects changes in forearm cutaneous sub-dermal microvascular blood flow during iontophoresis of topically applied vasoactive drugs. The protocol employed for each patient is identical and is described below.

Iontophoresis

This is a non-invasive technique facilitating the delivery of relatively small charged ions into local tissue. In the protocol, sodium nitroprusside (SNP) was used to measure the endothelium-independent response and acetylcholine (ACh) the endothelium-dependent response. 1% solutions of these drugs were made up by dissolving 0.1g of solute in 10ml of 0.5%saline solution. These solutions were than placed into a specially designed perspex chambers, which were applied to the forearm extensor surface using adhesive tape. Care was taken in the placement of these chambers to avoid hair, broken skin and superficial veins. A thin platinum wire on the



inside surface of these plastic chambers was connected by means of an external wire to a source of constant current. SNP, containing the negatively charged nitroprusside ions, was placed in the cathodal chamber. The ACh solution, containing the positively charged ACh ions, was placed in the anodal chamber.

When the battery-operated constant current source is activated, the positive charge at the anode repels the positively charged ACh ions into the superficial layers of the skin, thereby effecting delivery of this vasoactive drug to the subdermal capillaries. The same process occurs at the cathode for the negatively charged SNP ions.

The Principle of Laser Doppler Imaging

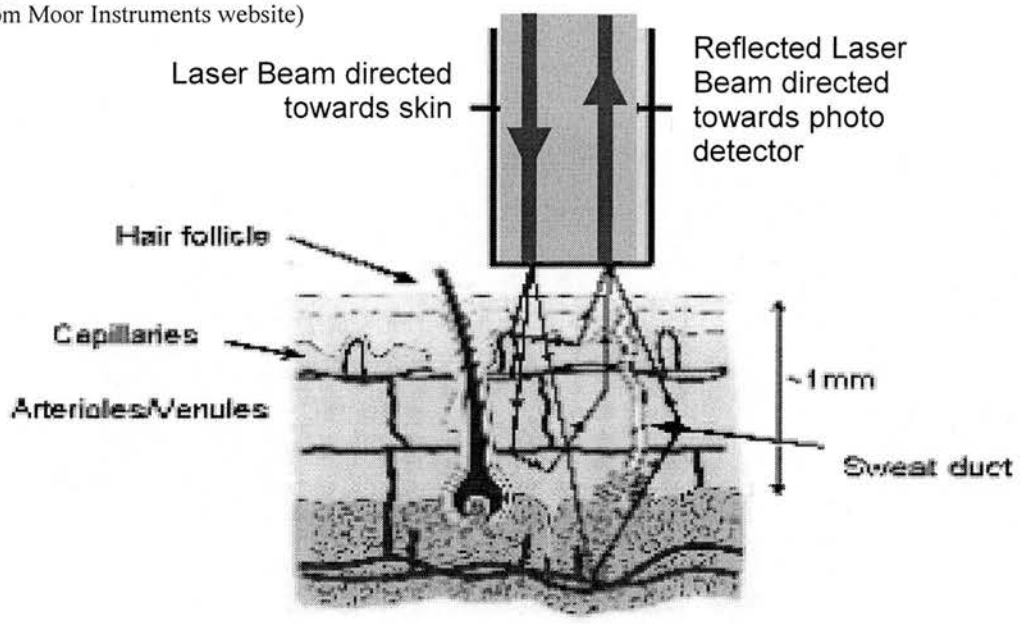
The Doppler principle implies that the wavelength of a reflected electromagnetic waveform is shifted if it is reflected off a moving object. Furthermore, this shift is proportional to the velocity of this moving object. This is the principle used by the laser Doppler imager to record changes in forearm cutaneous perfusion during iontophoresis. The laser Doppler imager used (Moor Instruments Ltd) made use of a red laser (wavelength 633nm, power 1mW, beam diameter 1mm).

A laser beam is directed towards a point on the skin. Subdermal capillary recruitment occurring during iontophoresis results in increasing numbers of mainly red blood cells in the superficial layers of the skin. A laser beam that reflects off one of these moving blood cells will exhibit a Doppler shift which is picked up by the detector section of the imager. The presence and magnitude of this shift therefore reflects a perfusion response to the vasoactive chemicals applied. This response can be monitored at a single point – this is the method applied by single point laser Doppler fluximetry.

Laser Doppler imaging, however, scans thousands of individual points over a given area (which corresponds to the area over which iontophoresis takes place). The area of interest (over the iontophoresis chambers) is defined manually and the laser Doppler imager scans over this preset area in a linear fashion. The Doppler shift at each point is recorded, and as several thousand points are scanned, this method averages the perfusion changes over an area. This, therefore, takes into account the heterogeneous nature of these perfusion changes over an area. Each scan takes approximately 50 seconds to scan over the area of skin on which the iontophoresis chambers are attached.

The tissue thickness penetrated by the laser is typically 1mm. Flow is measured in capillaries with diameters in the order of 10 microns with flow velocities ranging from typically 0.01 to 10mm/s. This is shown in Figure 1 below.

Figure 5.1:
Schematic of superficial skin layer
demonstrating blood vessels scanned by LDI.
(Adapted from Moor Instruments website)



The laser Doppler imager derives the perfusion change at each point from the Doppler shift recorded at this point - this is recorded as an arbitrary unit. The average change in perfusion is calculated by taking each point lying in the area beneath the anode or cathode chambers and calculating the median for this area. This gives a single number as an arbitrary unit to describe the perfusion in the area of interest during each scan. The median is used because the Doppler shifts recorded at each point within the area of interest are not normally distributed.

The Iontophoresis Protocol

This works in conjunction with the laser Doppler imager. A total of 20 scans are made by the imager and the current is varied for each of these scans as shown in the table below. An incremental current regime is used to increase drug delivery throughout the 20 scans reaching a peak at scan 15, after which the current is withdrawn, drug delivery is stopped and perfusion changes regress. Each scan takes approximately 50 seconds to complete.

A baseline scan is performed before any current is applied. After this the current is increased to 5μAmp for the next 4 scans. A further increase to 10μAmp for 4 scans and then 15μAmp for a further 4 scans is followed by the maximum current of 20μAmp for the next 2 scans. After this the current is discontinued and another 5 scans undertaken to record residual perfusion changes.

Scan No.	Current
1	0
2	5
3	5
4	5
5	5
6	10
7	10
8	10
9	10
10	15
11	15
12	15
13	15
14	20
15	20
16	0
17	0
18	0
19	0
20	0

At the anode, the ACh response is usually maximal at scan 16 following the maximal current. The SNP response at the cathode can be more sustained with increases in perfusion sometimes continuing even after the current has been stopped. Voltage is recorded during each scan to facilitate the calculation of the resistance time integral.

The median perfusion value is calculated for each of the 20 scans for the area of interest. This can be plotted as a graph and the total perfusion response can be derived for the area under the curve (AUC) for this graph.

The Hyperaemic Response

It has been noted that Laser Doppler imaging during iontophoresis sometimes produces an artefact known as the hyperaemic response, which may over-estimate the perfusion response. This usually occurs at the cathode especially in subjects with high skin resistance, reflected by high measured voltages in the circuit. This artefact consists of a vasodilating response picked up by the laser Doppler imager under these circumstances. If it does occur, it can hamper the interpretation of the SNP response, as vasodilation that occurs is not a direct effect of iontophoresis of SNP ions.

It is thought that the hyperaemic response is a local neurally-mediated response which occurs as a result of the high voltages needed to sustain the current under conditions of high skin resistance. Several factors are thought to be important in determining whether a hyperaemic response is elicited. These include:

- Solution vehicle used
- Area of iontophoresis chamber
- Cumulative charge used

We used iontophoresis chambers with a large surface area and a low resistance vehicle solution, which is known to minimise this artefact (14). In order to identify which scans were subject to this artefact, laser Doppler imaging was undertaken during saline solution (0.75%) iontophoresis, so that no vasoactive drugs were being delivered. 0.75% saline was chosen in order to mimic the ionic concentration of the drug solutions. In fact, the ionic concentration of the drug solutions used is 1.5% so a control solution of 0.75% is conservative. This figure of 0.75% was chosen because it was recognised from previous work that this concentration of saline produces resistance values similar to that of the drug solution used during iontophoresis. In practice, one is more likely to encounter artefact responses with a lower concentration solution and so using a control solution of 0.75% ought to provide an over-estimate of the artefact response produced.

It had been thought, anecdotally, that subjects in whom a hyperaemic response was noted, had high cutaneous resistance thereby necessitating larger voltages in order to keep the current delivered at the level shown in the iontophoresis protocol. Because skin resistance is dynamic throughout a scan, the resistance-time integral (RTI) was calculated for each of these scans using recorded voltages. The RTI is therefore a reflection of the resistance for the duration of each scan.

The chart below shows the perfusion response during iontophoresis of 0.75% saline at the anode and the cathode against the RTI. It is evident that in the 84 vehicle scans performed there is generally a low response with the vast majority being less than 300 units. Only 7% of control scan perfusion responses exceeded this value. Generally there is a higher response to the control solution at the cathode. The exact reason for this is unclear. There is no clear correlation between the RTI and the perfusion response at either the anode or the cathode. Those scans with the highest RTI did not produce the highest perfusion responses and conversely, the subjects in which the biggest perfusion responses were seen, did not have the largest RTI. No clear pattern has emerged linking perfusion responses to the magnitude of the RTI, and it is likely that other factors, as yet unknown, play a role in producing this artefact. However, all recognised variables that produce this artefact were adjusted to keep it to a minimum (14).

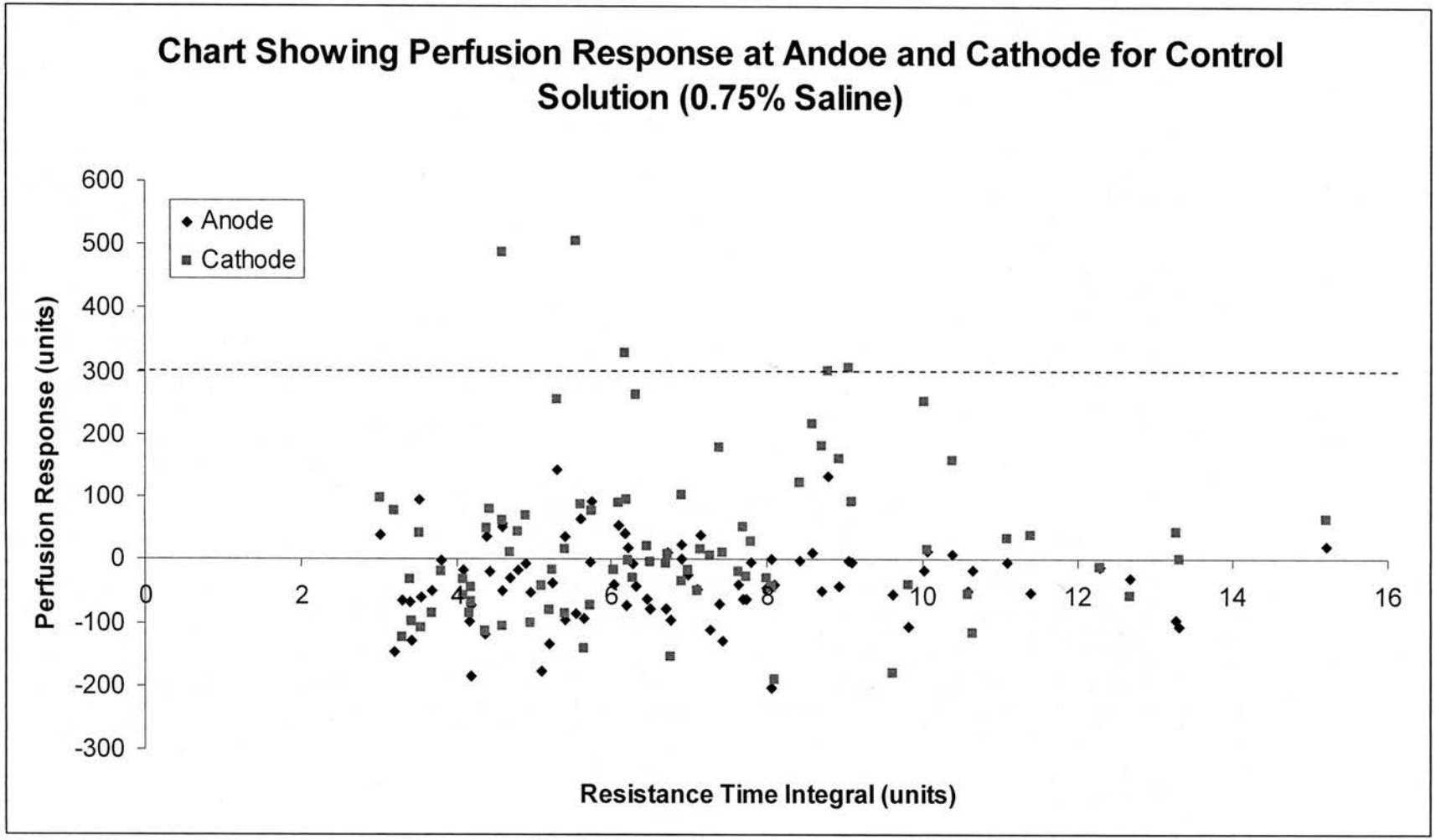


Chart Showing Perfusion Response at Andoe and Cathode for Control Solution (0.75% Saline)
Anode
Cathode
Perfusion Response (units)
600
500
400
300
200
100
0
-100
-200
-300
0
2
4
6
8
10
12
14
16
Resistance Time Integral (units)

Calculating the Perfusion Response from Laser Doppler Imaging

The data obtained from the laser Doppler imager consists of 20 images of the area scanned (one image corresponding to one scan at each of the incremental iontophoresis points). Each pixel within the scans corresponds to one point at which the flux in the wavelength of the reflected laser beam is measured.

Using a software package from Moor instruments, supplied with the laser Doppler imager, the area of interest (AOI) is marked for each of these scans. The AOI is the circular area within the ACh or SNP chambers, depending on which response is being analysed. Each pixel within the AOI is assigned a number (arbitary unit) depending on the wavelength flux at this point. All the values within the AOI are collected in this way and a median value is assigned to represent the perfusion response. In this manner, 20 values are obtained for the ACh and SNP response for each scan. These numbers can be represented graphically with the cumulative perfusion response being calculated by the area under the curve (AUC).

The values derived from the LDI to reflect microvascular responsiveness can be presented in several ways. Here the data for each scan are presented in 4 different ways :

- Raw AUC value (AUC)
- AUC value corrected for RTI (AUC-r)
- Raw AUC value – baseline (AUC-b)
- Raw AUC value – baseline corrected for RTI (AUC-br)

It is not known which measure of perfusion response is most valid and the data on the reproducibility of the scans presented at the end of this chapter should go some way to addressing this question.

Correcting for Skin Resistance

The amount of drug delivered during iontophoresis depends on :

- The magnitude of the current applied to the circuit by the power source
- The resistance of the skin (higher skin resistance leads to reduced drug delivery for a given current)

The current is increased incrementally from 5μAmp to 20μAmp over a 15 minute period during which the laser Doppler imager scans over the area of the skin to record changes in perfusion. The voltage within the circuit is monitored during the scan and the resistance calculated using Ohm's Law :

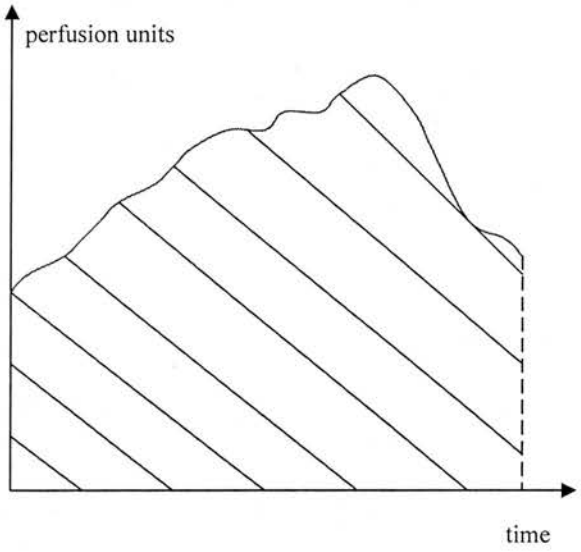
$$\text{Voltage} = \text{current} \times \text{resistance}$$

However, the skin resistance is dynamic and starts off relatively high at the start, reducing constantly during iontophoresis. Resistance within the circuit is therefore measured at regular intervals and the graph of resistance against time is plotted for each scan. The area under this curve (AUC) serves as an index of the skin resistance for this period and is referred to as the resistance time integral (RTI). The changes in perfusion measured by the Laser Doppler imager during iontophoresis can be corrected for by the RTI to allow for the differences in drug delivery caused by variation in skin resistance (15).

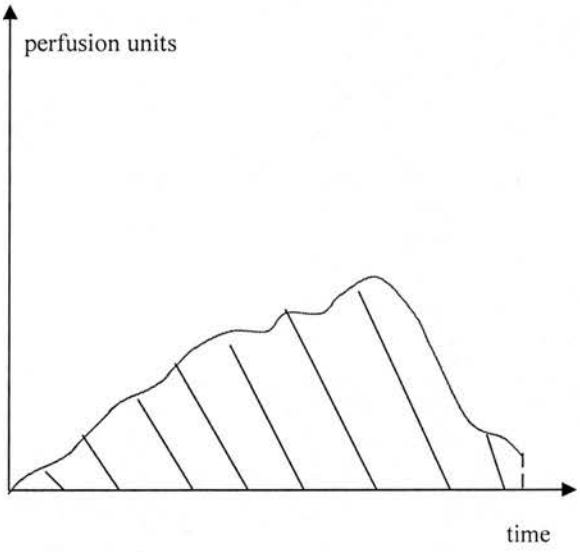
Subtracting the Baseline Value

The AUC response derived from the LDI gives the raw-AUC value. The baseline value at the first scan (0μAmp) can be deducted from each subsequent scan so that each of the 19 subsequent scans reflect the perfusion response to iontophoresis. This will ensure that an artificially high response is not derived as a result of a high baseline result. However, it is not known whether this approach is valid or whether the total AUC response is a better guide to the vascular response.

Graph showing raw-AUC response



Graph showing AUC-baseline response



The line graph represents the perfusion response against time. The shaded area represents the area under the curve. The graph on the right shows the AUC corrected for the baseline (i.e. the entire graph is shifted downwards so that the initial perfusion value is 0)

If this approach is adopted this AUC-baseline response may still be multiplied by the resistance time integral (RTI) to correct for skin resistance and differences in drug delivery.

Reproducibility of the Perfusion Response using LDI

Inter-arm Variation

Little information exists about the reproducibility of LDI results on different parts of the body. Most investigators use the forearm as a relatively flat accessible and easy to use region. However, it is unknown how even opposite arms differ in their superficial cutaneous perfusion response to the iontophoresis of vasoactive drugs. In an attempt to characterise this, iontophoresis and laser Doppler imaging was performed on both arms consecutively using ACh and SNP. This was done on 24 healthy control subjects and 4 'Syndrome X' patients at baseline and 12 'Syndrome X' patients after treatment with either placebo or metformin, giving a total of 40 subjects in whom data on perfusion response in both arms on the same session are available.

ACh

Right Arm

AUC	AUC-r	AUC-b	AUC-br
1848	8760	1044	4949
3805	25912	3148	21438
1643	10417	839	5319
2479	12469	1714	8621
3480	19349	2872	15968
1137	8641	656	4986
1366	9234	729	4928
2650	8878	2062	6908
2971	13162	2186	9684
2896	19345	2180	14562
1722	19235	957	10690
4485	24623	3606	19797
2011	10759	1060	5671
2426	13222	1583	8627
1938	11066	1213	6926
760	5715	221	1662
2139	15144	1384	9799
1325	8599	688	4465
2508	17054	1547	10520
1600	9152	923	5280
1545	7431	849	4084
2307	14649	1582	10046
2525	10959	1770	7682
3210	19132	2240	13350
1380	8416	752	4586
973	6568	296	1998
1881	10816	1126	6475
1242	6819	642	3525
1464	7071	719	3473
1767	12298	924	6391
2934	16636	2326	13188
1390	8590	537	3319
1476	6539	858	3801
1363	5779	520	2205
2624	10207	1908	7422
1485	6074	534	2184
1459	9119	871	5444
1624	8494	927	4848
1419	6939	645	3154
857	4928	73	420

Left Arm

AUC	AUC-r	AUC-b	AUC-br
1775	8698	1010	4949
3189	17571	2621	14442
1412	8881	677	4258
1680	7375	964	4232
3445	18086	2769	14537
1078	7956	411	3033
2050	11583	1432	8091
3438	9833	2448	7001
4870	23766	4007	19554
2829	16550	2065	12080
2115	17533	1281	10619
4357	23484	3484	18779
2213	14871	1292	8682
3531	17196	2364	11513
2320	11855	1535	7844
954	7365	376	2903
3003	16727	2081	11591
1261	9470	644	4836
4467	22648	3232	16386
1506	13810	741	6795
1627	10022	872	5372
2148	15100	1256	8830
2723	14895	1968	10765
2722	15216	2055	11487
1607	11088	882	6086
971	7749	167	1333
1579	6032	814	3109
1302	12239	702	6599
1063	11587	259	2823
1384	10851	629	4931
1122	4825	504	2167
1492	9250	521	3230
1183	6270	428	2268
1010	7373	118	861
2762	10496	1850	7030
1258	7171	591	3369
1756	14926	1168	9928
1317	7507	532	3032
1173	6686	614	3500
1124	7531	290	1943

SNP

Right Arm

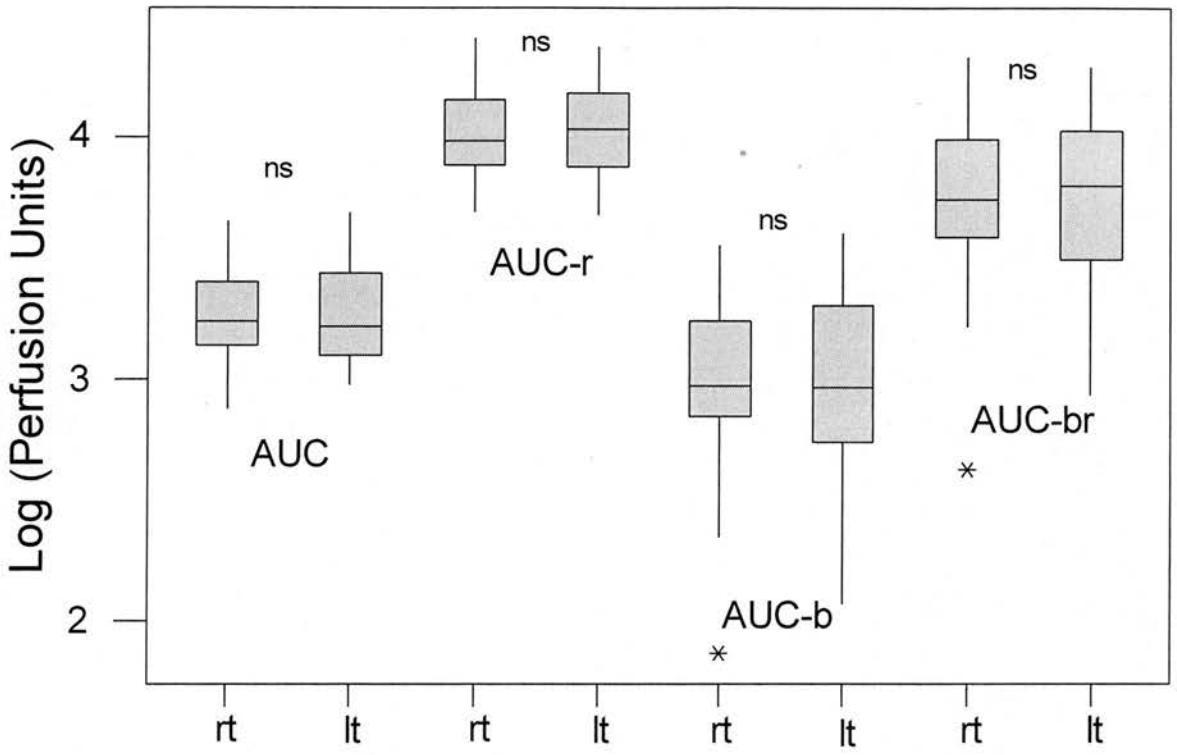
AUC	AUC-r	AUC-b	AUC-br
2566	12163	1821	8632
3416	23263	2652	18060
1534	9726	857	5433
2225	11192	1353	6806
2144	11921	1536	8540
1519	11544	989	7516
1802	12182	978	6611
2816	9434	2150	7203
3555	15749	2790	12360
1078	7201	352	2351
2193	24496	1252	13985
2922	16042	2078	11408
2761	14771	1790	9577
1405	7657	543	2959
2532	14458	1846	10541
1190	8949	631	4745
1492	10563	816	5777
2116	13733	1498	9722
2371	16123	1351	9187
1137	6504	373	2134
782	3761	105	505
1926	12230	1132	7188
1122	4869	524	2274
1376	8201	532	3171
858	5233	201	1226
951	6419	246	1661
1298	7464	602	3462
1122	6160	605	3321
1374	6636	746	3603
1322	9201	518	3605
2129	12071	1433	8125
1211	7484	476	2942
1009	4470	431	1909
2425	10282	1690	7166
2341	9106	1508	5866
1359	5558	457	1869
1418	8863	820	5125
1936	10125	1240	6485
732	3579	-13	-64
1113	6400	260	1495

Left Arm

AUC	AUC-r	AUC-b	AUC-br
2201	10785	1455	7130
3089	17020	2481	13670
1770	11133	1083	6812
1766	7753	1079	4737
2009	10547	1391	7303
1750	12915	1014	7483
1839	10390	1172	6622
2396	6853	1513	4327
3464	16904	2660	12981
991	5797	246	1439
2106	17459	1263	10470
3949	21285	3057	16477
2889	19414	1938	13023
3018	14698	1890	9204
2369	12106	1604	8196
681	5257	152	1173
2010	11196	1196	6662
1703	12790	1017	7638
2764	14013	1754	8893
2740	25126	1877	17212
1770	10903	1113	6856
3081	21659	2424	17041
1340	7330	654	3577
1414	7904	806	4506
1273	8784	518	3574
1711	13654	887	7078
863	3297	20	76
1243	11684	727	6834
924	10072	189	2060
772	6052	7	55
2463	10591	1580	6794
1375	8525	425	2635
806	4272	81	429
1062	7753	288	2102
2369	9002	1497	5689
1017	5797	321	1830
2263	19236	1684	14314
1144	6521	448	2554
816	4651	130	741
1305	8744	590	3953

The distribution of the LDI perfusion units is non-parametric. Logarithmically transforming the data produces normality and allows comparison using the paired t-test. Boxplots of these data are shown below.

Boxplot of Inter-arm Variability of LDI response to ACh
(Log Transformed)



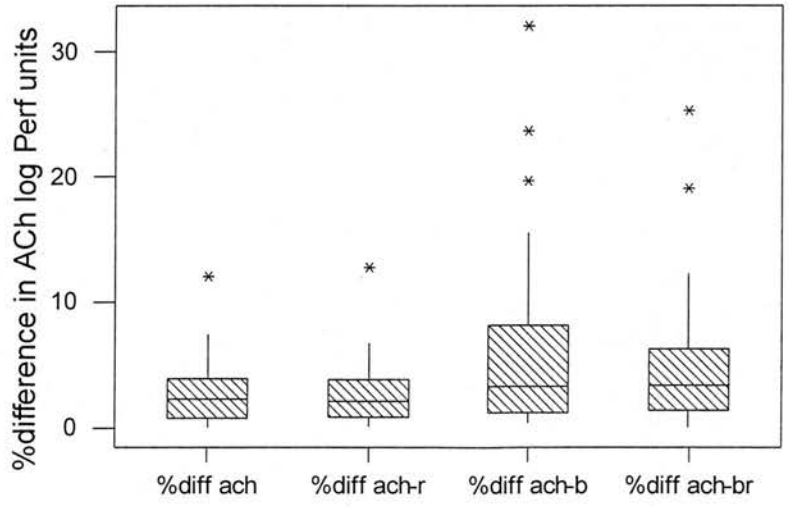
Paired T-Test Results (log transformed)

AUC	T-Value = -0.01	P-Value = 0.998
AUC-r	T-Value = -0.65	P-Value = 0.518
AUC-b	T-Value = 0.35	P-Value = 0.730
AUC-br	T-Value = 0.02	P-Value = 0.985

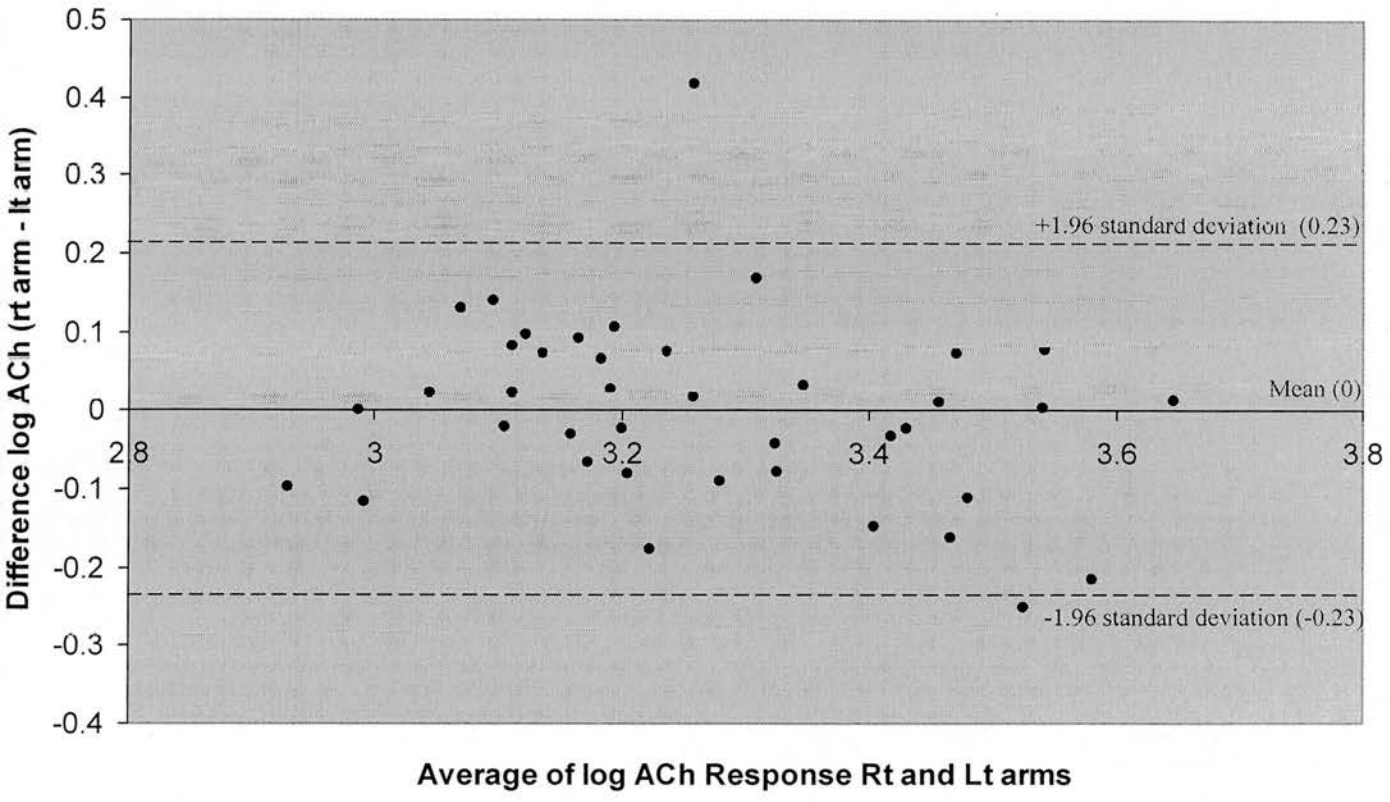
Average % diff between arms (log)

AUC	2.7%
AUC-r	2.7%
AUC-b	5.9%
AUC-br	4.7%

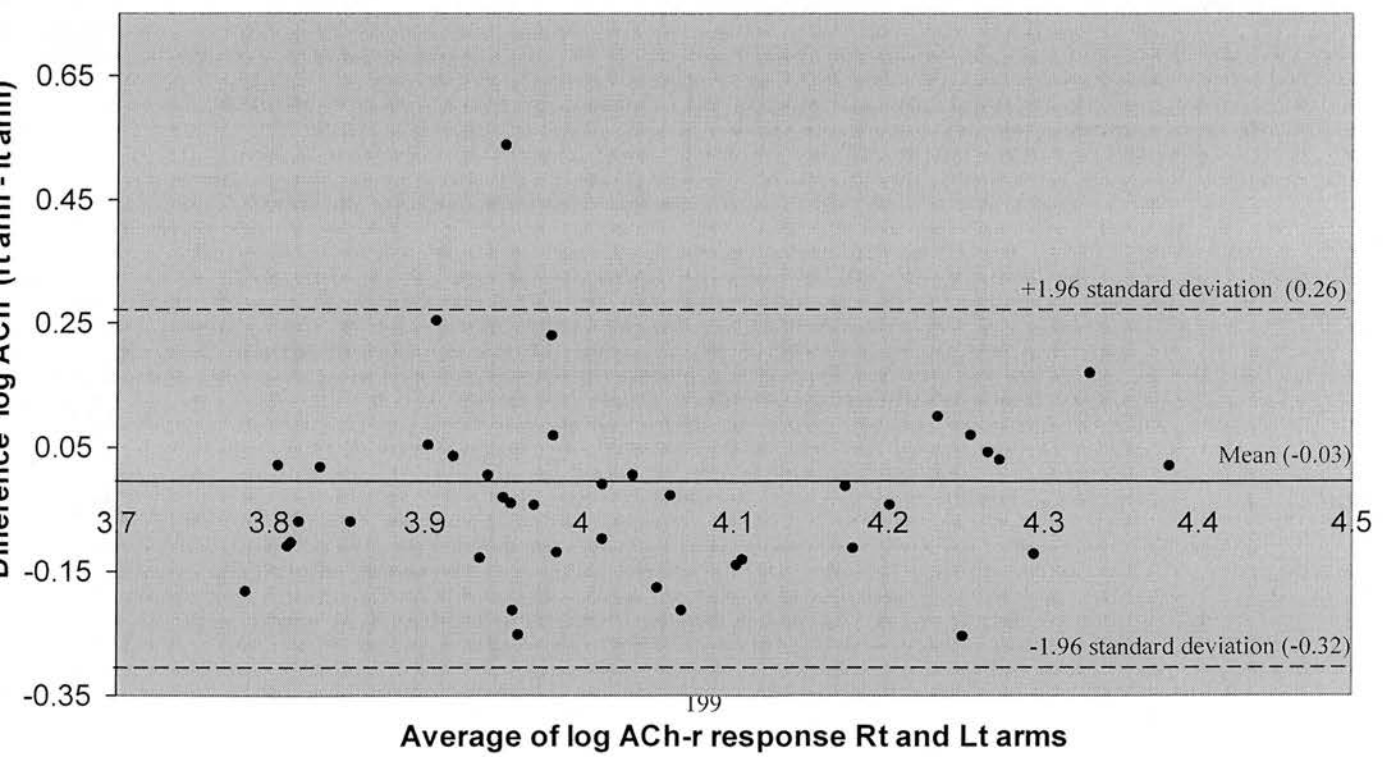
Boxplot of Inter-arm % Difference of LDI response to ACh
(Log Perfusion Units)



Bland Altman Plot of log ACh Perfusion Response

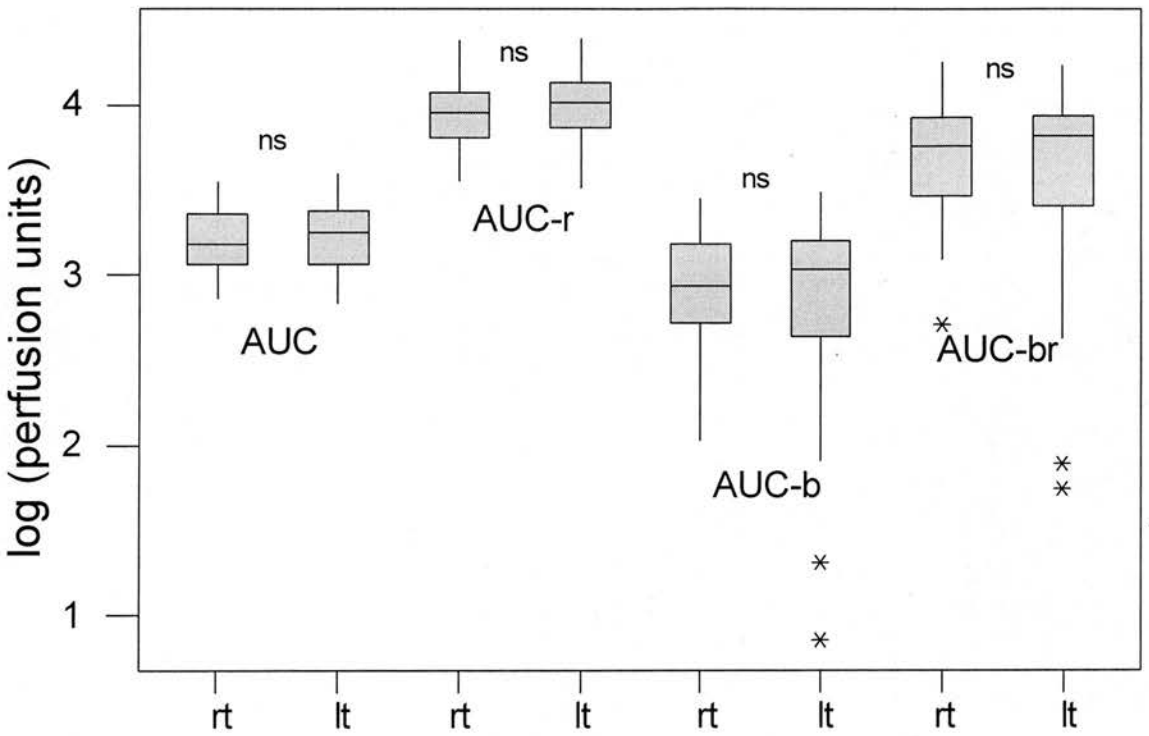


Bland Altman Plot of log ACh-r Perfusion Response



Boxplot of Inter-arm Variability of LDI response to SNP

(Log Transformed)



Paired T-Test Results (log transformed)

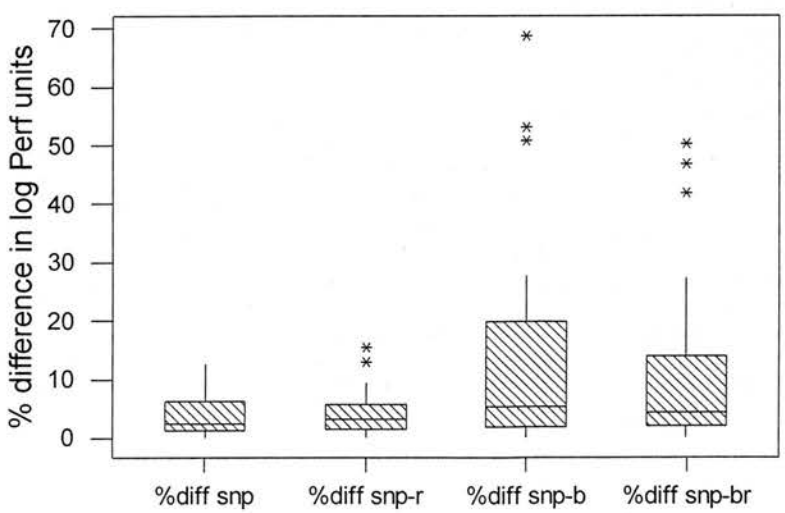
AUC	T-Value = -0.36	P-Value = 0.717
AUC-r	T-Value = -0.91	P-Value = 0.365
AUC-b	T-Value = 0.73	P-Value = 0.468
AUC-br	T-Value = 0.50	P-Value = 0.618

Average % diff between arms (log)

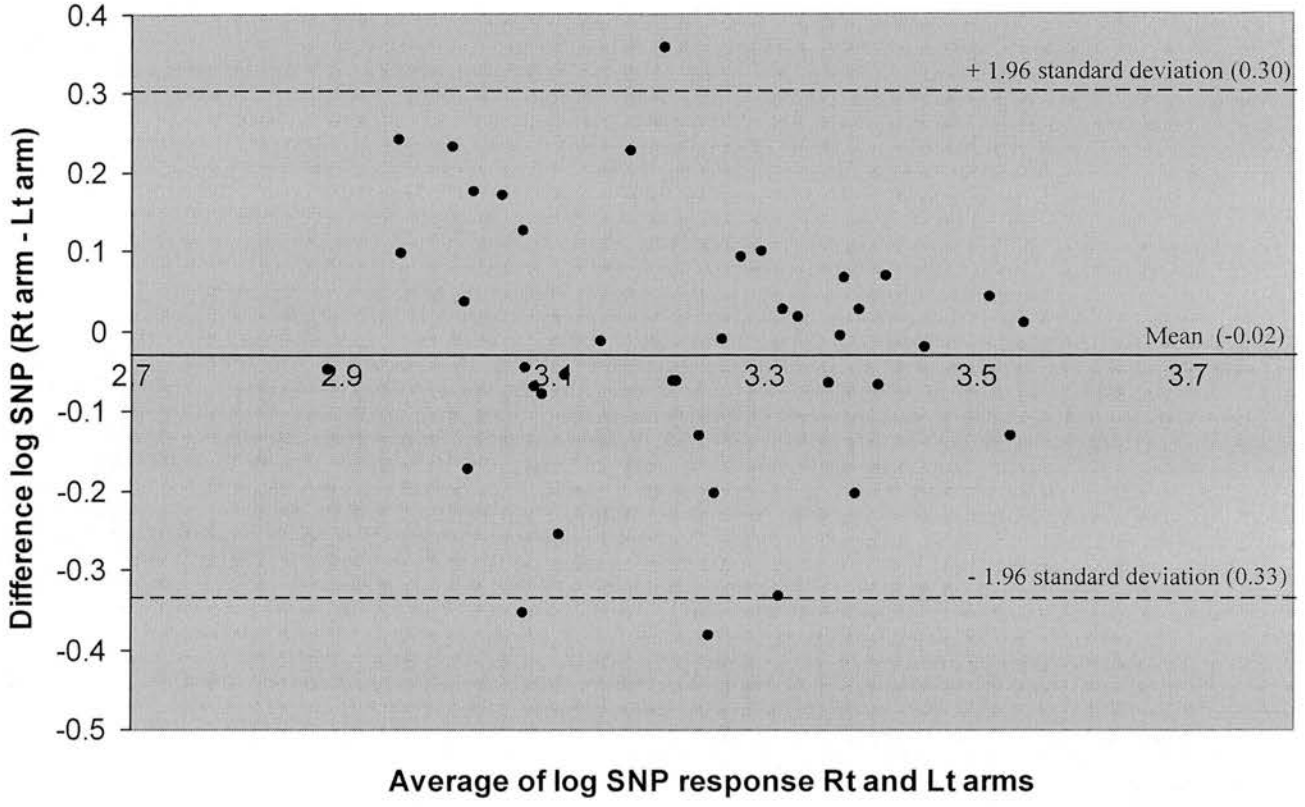
AUC	3.9%
AUC-r	3.8%
AUC-b	12.2%
AUC-br	9.9%

Boxplot of Inter-arm % Difference of LDI response to SNP

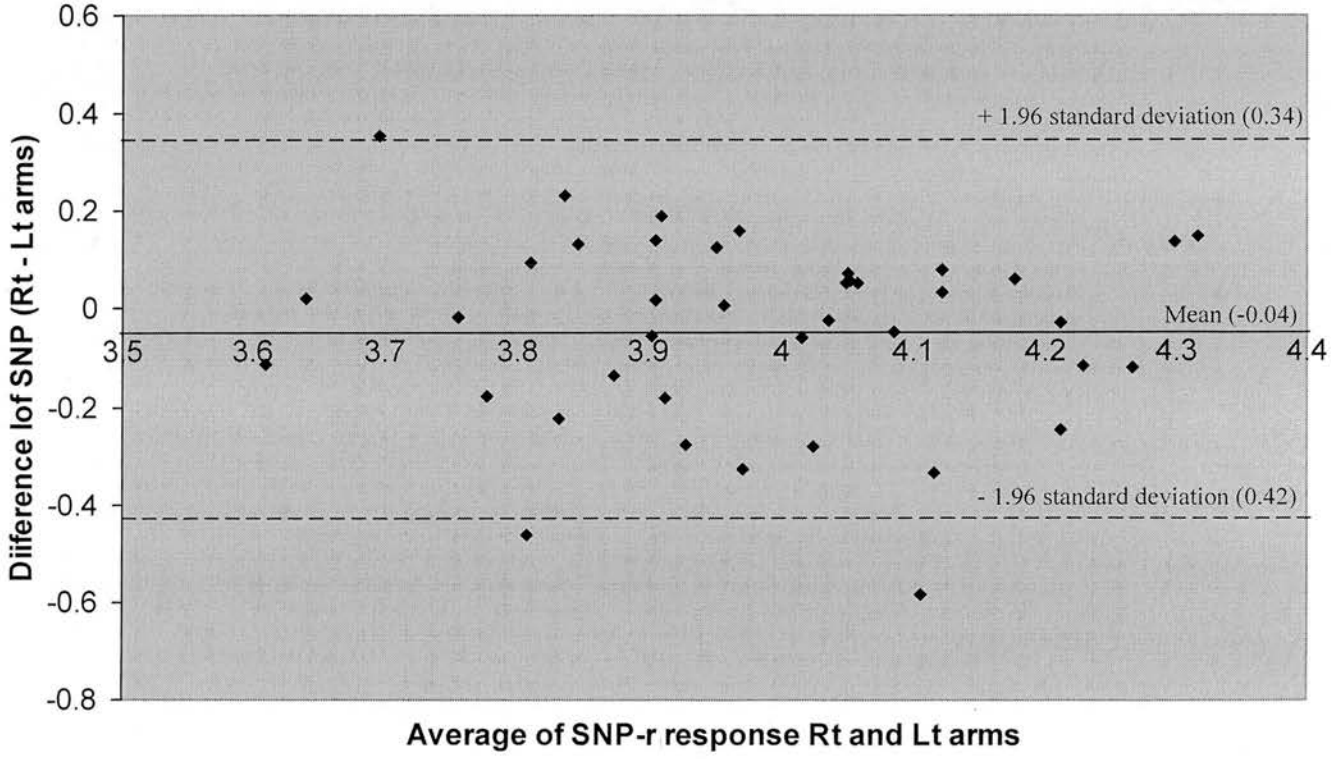
Log Perfusion Units



Bland Altman Plot of log SNP Perfusion Response



Bland Altman Plot of SNP-r Perfusion Response



Analysis of Inter-arm Variation

Boxplots above show that there is no significant difference between the measurements of LDI perfusion measurements taken from the left and right arms using the unpaired t-test for both the ACh and SNP response. This applies to all 4 methods of analysis including the raw area under the curve value (AUC), the value adjusted for the baseline value (AUC-b), the value adjusted for the resistance-time integral (AUC-r) and the value adjusted for both (AUC-br). However, it is clear looking at the boxplots, that the AUC and the AUC-r values have a smaller range than AUC-b and AUC-br reflecting less variation in the inter-arm measurements.

This is supported by the data looking at the mean % difference in the inter-arm results. AUC and AUC-r have a much lower % difference between arms. For the ACh response this is 2.7% and for the SNP response it is a little higher at 3.9% and 3.8% respectively.

The Bland-Altman graphs are used to look at the repeatability of the LDI measurements for both arms. Here the difference between the arm measurements are plotted against the average of both arms value. These are plotted for both the raw AUC and the AUC-r data for both the ACh and SNP response.

The mean difference for all 4 of the plots is approximately 0 as would be expected if the inter-arm measurements are approximately the same. The standard deviation of the differences is also calculated and shown in the plots as a dashed line. If good statistical repeatability is present then at least 95% of the differences ought to lie between 2 standard deviations (16).

For the ACh AUC measurement 2 data points out of 40 (5%) lie outside the 95% range suggesting that the technique meets the standard for repeatability. The ACh AUC-r measurement is better, however, with only 1 out of 40 difference (2.5%) lying

outside the 2 standard deviations of difference. The SNP repeatability is less good with 92.5% of the differences lying with 2 standard deviations of difference.

Looking at the spread of the data points on the Bland-Altman graph, magnitude of the ACh and SNP response analysed as both the raw AUC data and the AUC-r, has no bearing on the difference seen between the arms.

All of this would tend to suggest that some of the correction of the raw AUC data for the baseline has a detrimental effect on the repeatability between arms, of laser Doppler imaging in measuring the microvascular response to both ACh and SNP. The most robust analysis to ensure good repeatability between arms would seem to be using the raw AUC data or correcting for resistance-time integral (AUC-r). The latter appears to be slightly better than just using the raw AUC data. The SNP measurements are less good in terms of repeatability than ACh and just fall outside the Bland-Altman criteria for good statistical repeatability. This short-fall may reflect the relatively small sample size of 40 subjects. The superior repeatability of the ACh data is reflected in the smaller standard deviation of differences seen in the Bland-Altman plots.

Temporal Variation

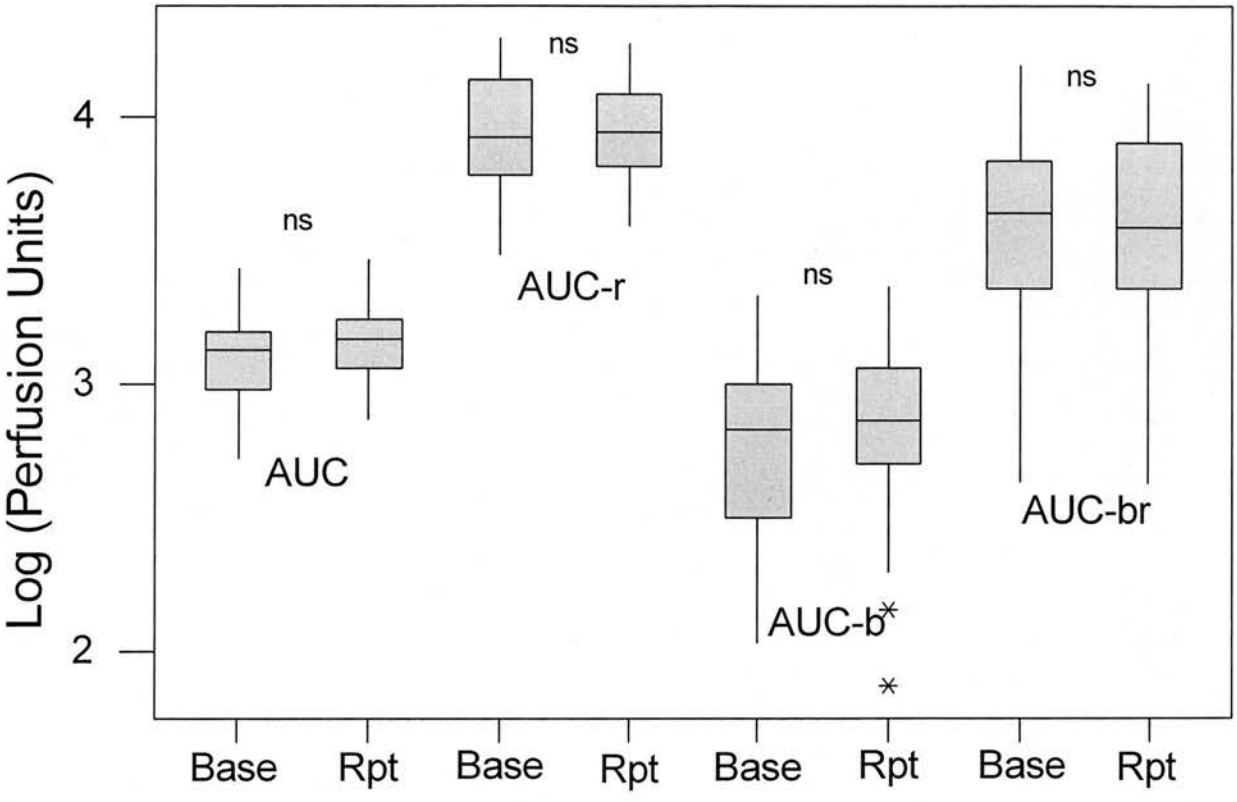
Many different factors affect the iontophoresis and laser Doppler perfusion response and although I have attempted to correct for and standardise as many of these as possible, there are likely to be additional factors as yet unknown and others for which one can only partly correct – such as the emotional state of the subject. It is not known how reproducible over time the perfusion response measured by LDI is.

In order to address this question, the LDI perfusion response was assessed in the 22 women with cardiac 'Syndrome X' who received placebo treatment, at baseline and again after 8 weeks. The raw data is shown in the table below:

	Baseline				*8 Weeks*			
ACh	**AUC**	**AUC-r**	**AUC-b**	**AUC-br**	**AUC**	**AUC-r**	**AUC-b**	**AUC-br**
	1400	11194	850	6796	2291	12005	1874	9820
	1105	15135	405	5547	1094	13938	461	5873
	2586	14264	1703	9394	2538	12995	1872	9585
	1388	7123	972	4988	1538	8597	654	3656
	1136	8889	319	2496	1213	9668	513	4089
	1351	6285	763	3550	759	3909	142	731
	1808	7503	1014	4208	1672	13493	1064	8586
	1647	8543	990	5135	1672	10918	878	5733
	1341	14179	469	4959	1464	7071	719	3473
	526	4651	134	1185	1463	7169	757	3709
	2699	20008	2121	15723	1529	11330	1068	7914
	815	5467	197	1321	1353	10188	735	5535
	776	5631	325	2358	1036	7107	242	1660
	864	4809	296	1648	1982	4995	1365	3440
	2462	18926	1707	13122	2485	18911	1760	13394
	1359	7366	673	3634	1173	5114	526	2293
	1212	12666	673	7033	2934	16636	2326	13188
	1051	10362	424	4180	734	6980	195	1854
	764	3078	107	431	1363	5779	520	2205
	1533	13939	1003	9117	1459	9119	871	5444
	1380	8416	752	4586	1419	6939	645	3154
	973	6568	296	1998	857	4928	73	420
SNP	954	7628	471	3766	1362	7137	978	5125
	1028	14080	294	4027	1035	13186	585	7453
	1340	7391	490	2703	1614	8264	914	4680
	886	4547	436	2237	1543	8625	659	3684
	1482	11596	598	4679	895	7133	295	2351
	761	3540	359	1670	1169	6020	444	2287
	2244	9312	1303	5407	1649	13307	992	8005
	1672	8673	1015	5265	939	6132	380	2481
	1008	10658	28	296	1374	6636	746	3603
	646	5712	263	2326	1649	8080	1022	5008
	1674	12410	1203	8918	1258	9322	856	6343
	692	4642	152	1020	871	6559	312	2349
	1484	10769	729	5290	893	6126	285	1955
	940	5232	410	2282	769	1938	93	234
	1541	11846	1022	7856	1208	9193	600	4566
	2749	14845	1729	9337	1464	6383	925	4033
	1004	10492	446	4661	2129	12071	1433	8125
	1370	13507	732	7217	573	5449	63	599
	1117	4500	587	2365	2425	10282	1690	7166
	1091	9917	502	4563	1418	8863	820	5125
	858	5233	201	1226	732	3579	-13	-64
	951	6419	246	1661	1113	6400	260	1495

Boxplot of Temporal Variability of LDI Response to ACh

(Log Transformed)



Paired T-Test Results (log transformed)

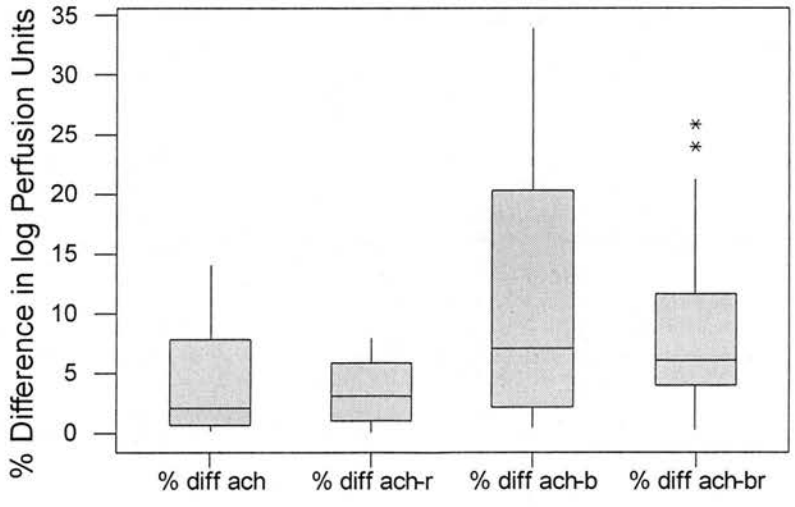
AUC	T-Value = -1.48	P-Value = 0.155
AUC-r	T-Value = 0.10	P-Value = 0.923
AUC-b	T-Value = -0.81	P-Value = 0.428
AUC-br	T-Value = -0.09	P-Value = 0.931

Average % diff (log)

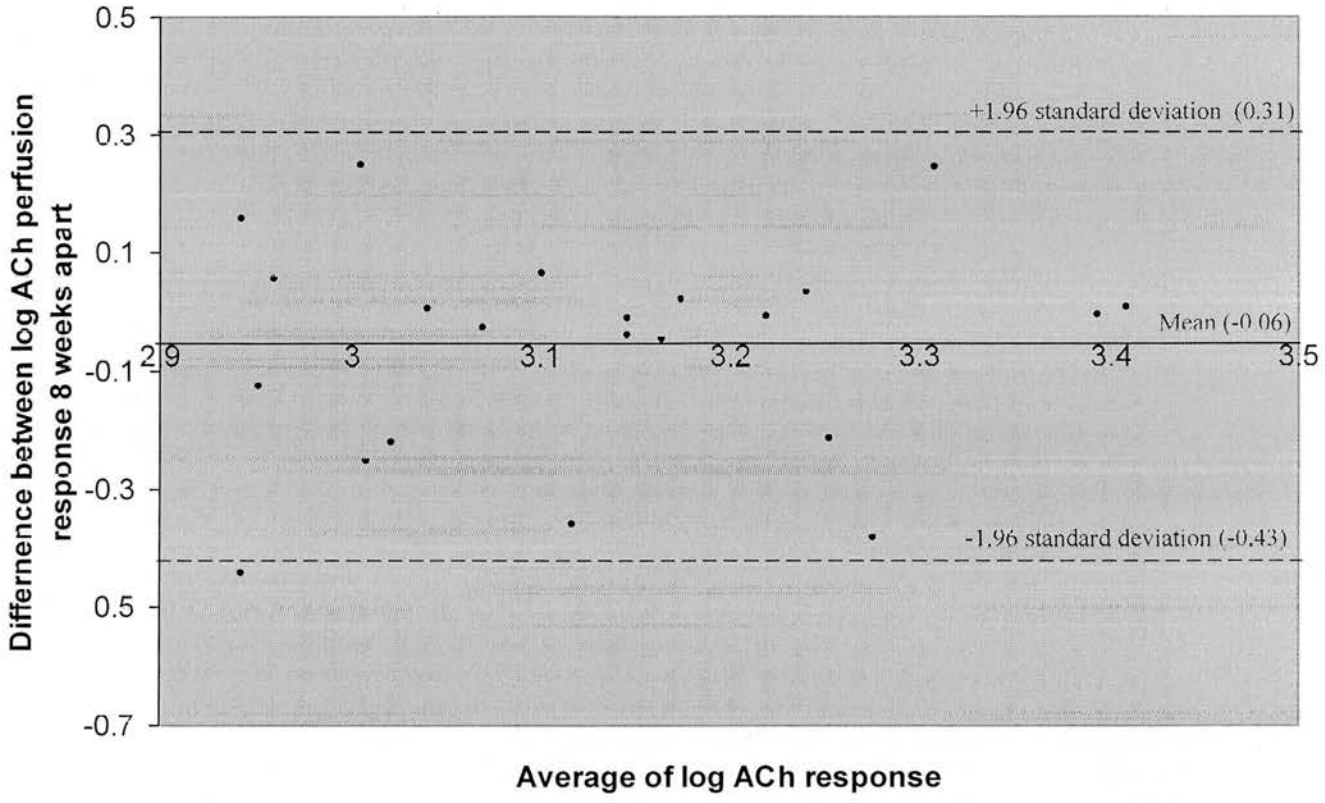
AUC	4.3%
AUC-r	3.5%
AUC-b	11.4%
AUC-br	8.4%

Boxplot of Temporal % Difference of LDI Response to ACh

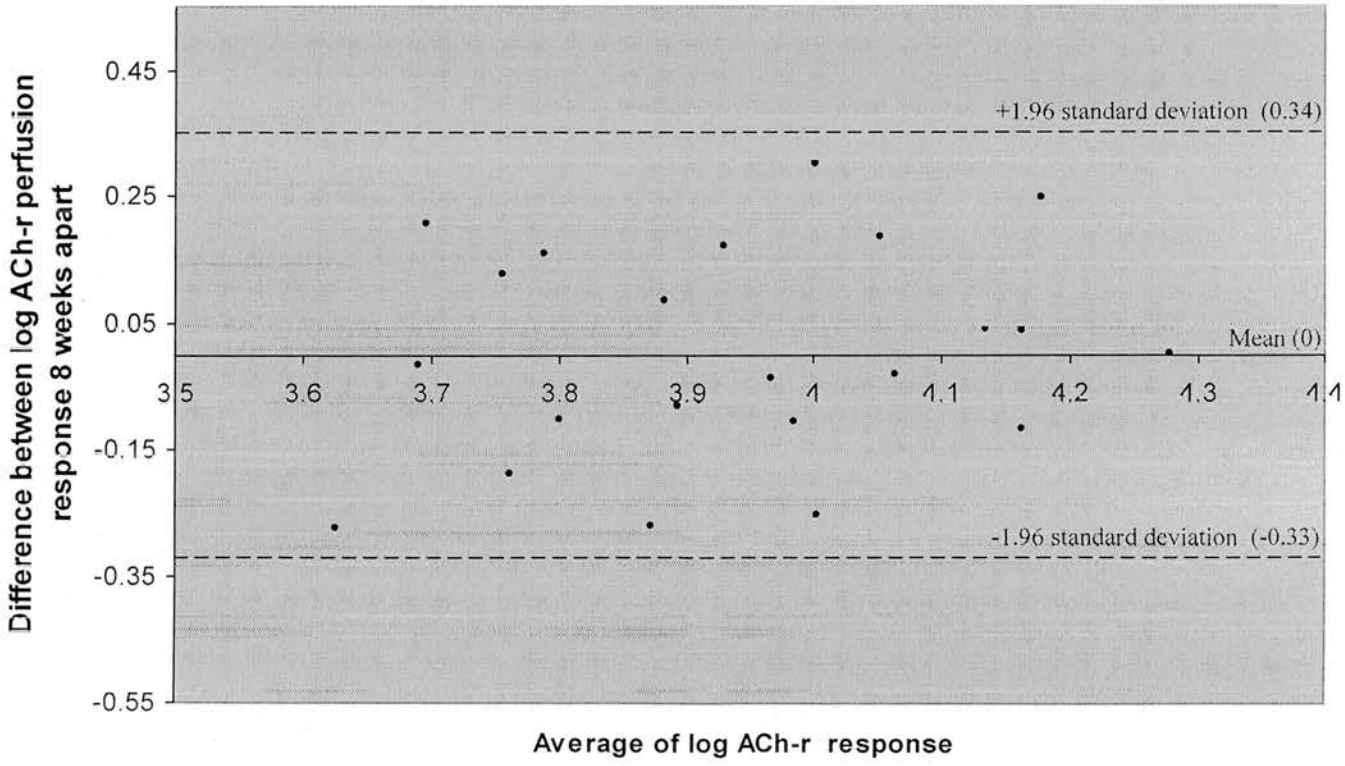
Log Perfusion Units



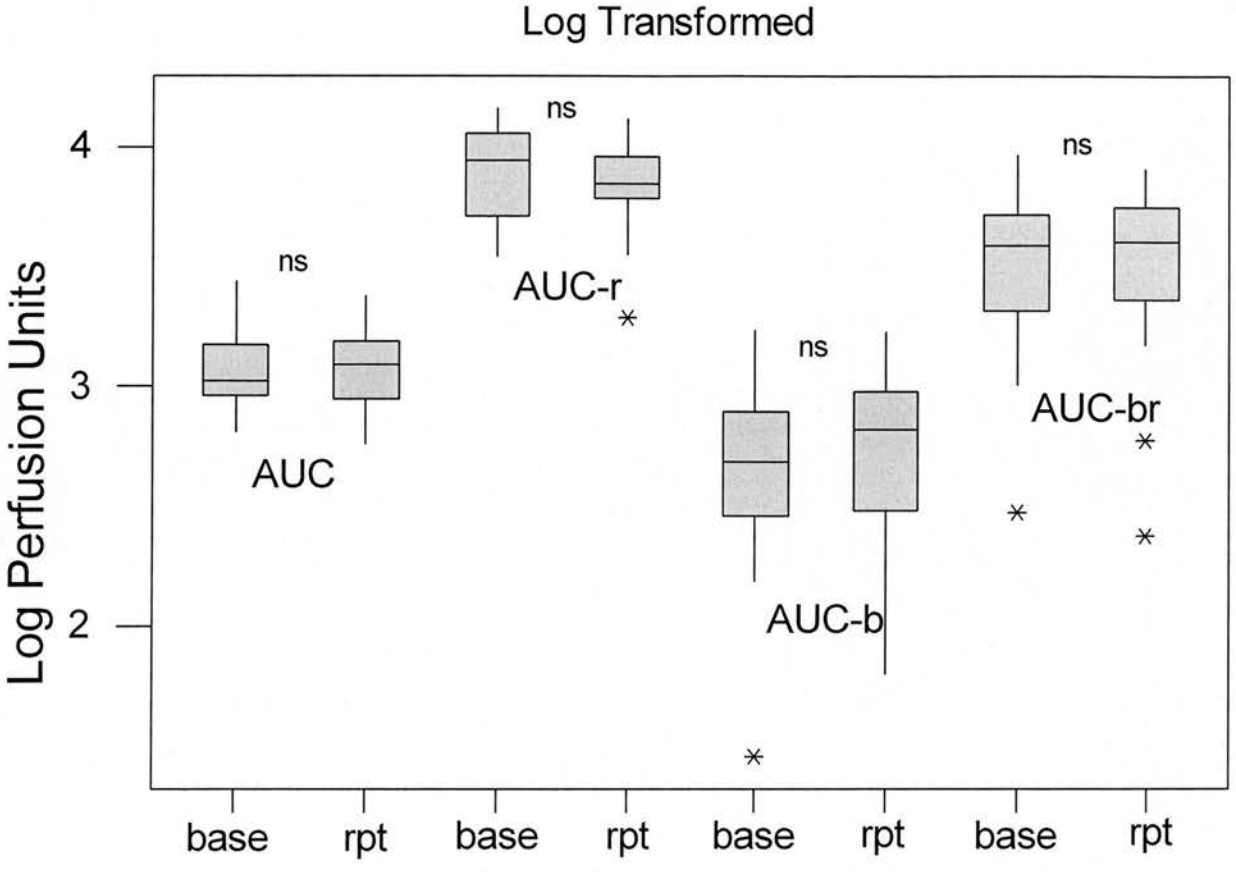
Bland Altman Plot of log ACh Response 8 Weeks Apart



Bland Altman Plot of lof ACh-r Perfusion Response 8 weeks Apart



Boxplot of Temporal Variability of LDI Response to SNP



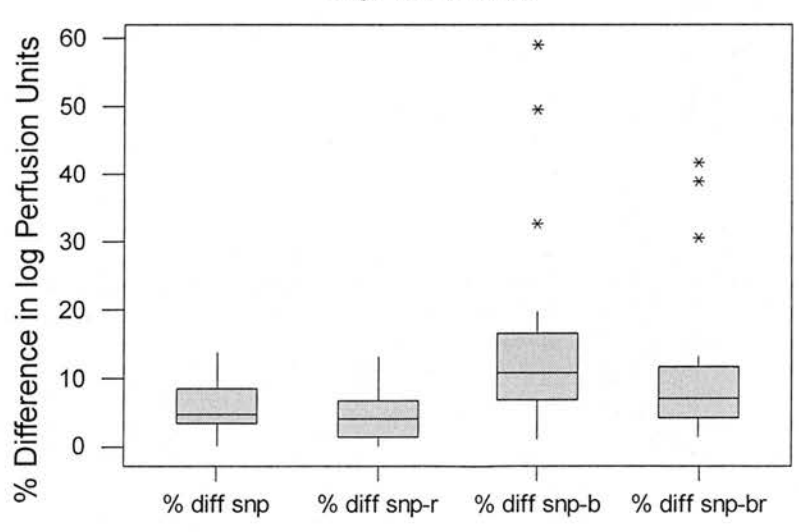
Paired T-Test Results (log transformed)

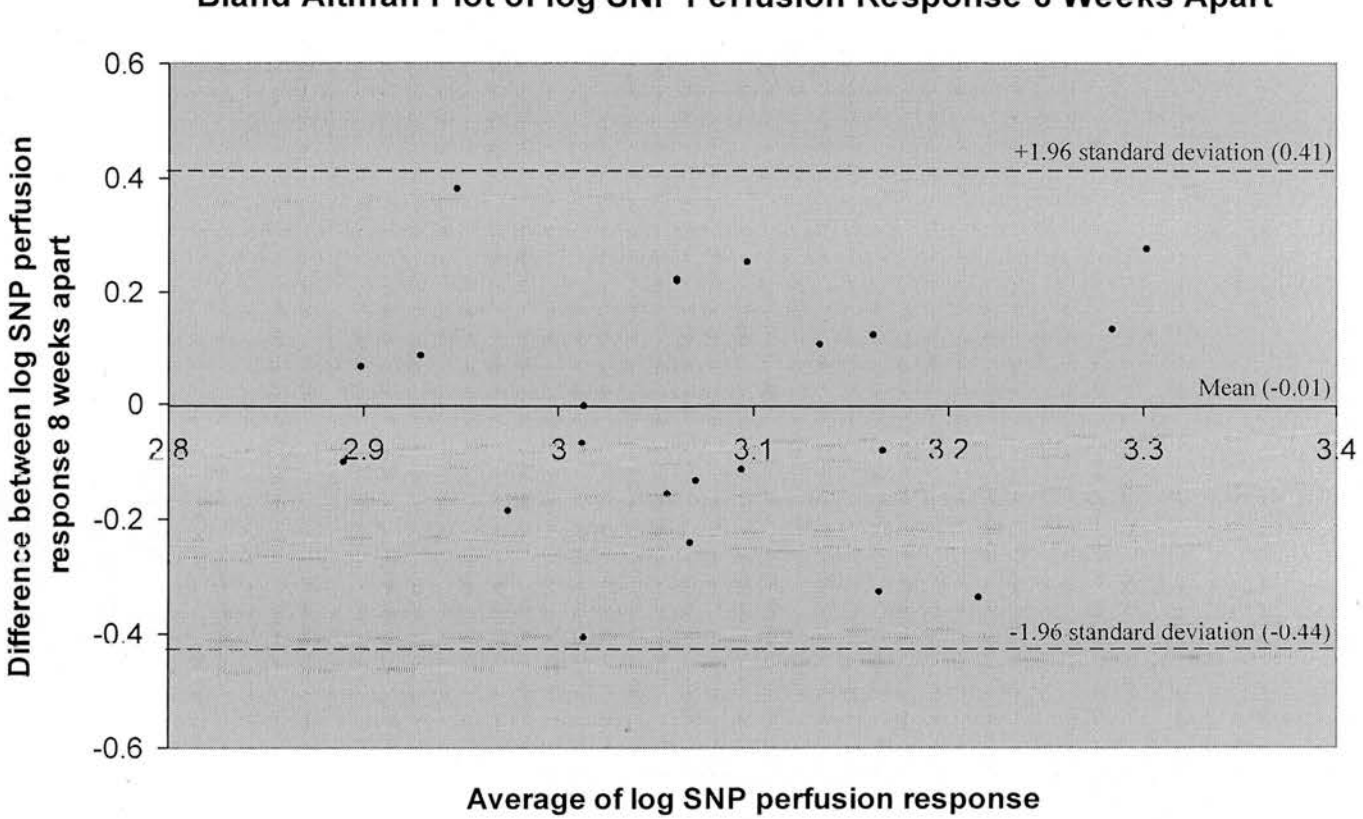
AUC	T-Value = -0.29	P-Value = 0.778
AUC-r	T-Value = 1.06	P-Value = 0.301
AUC-b	T-Value = -0.45	P-Value = 0.658
AUC-br	T-Value = 0.09	P-Value = 0.928

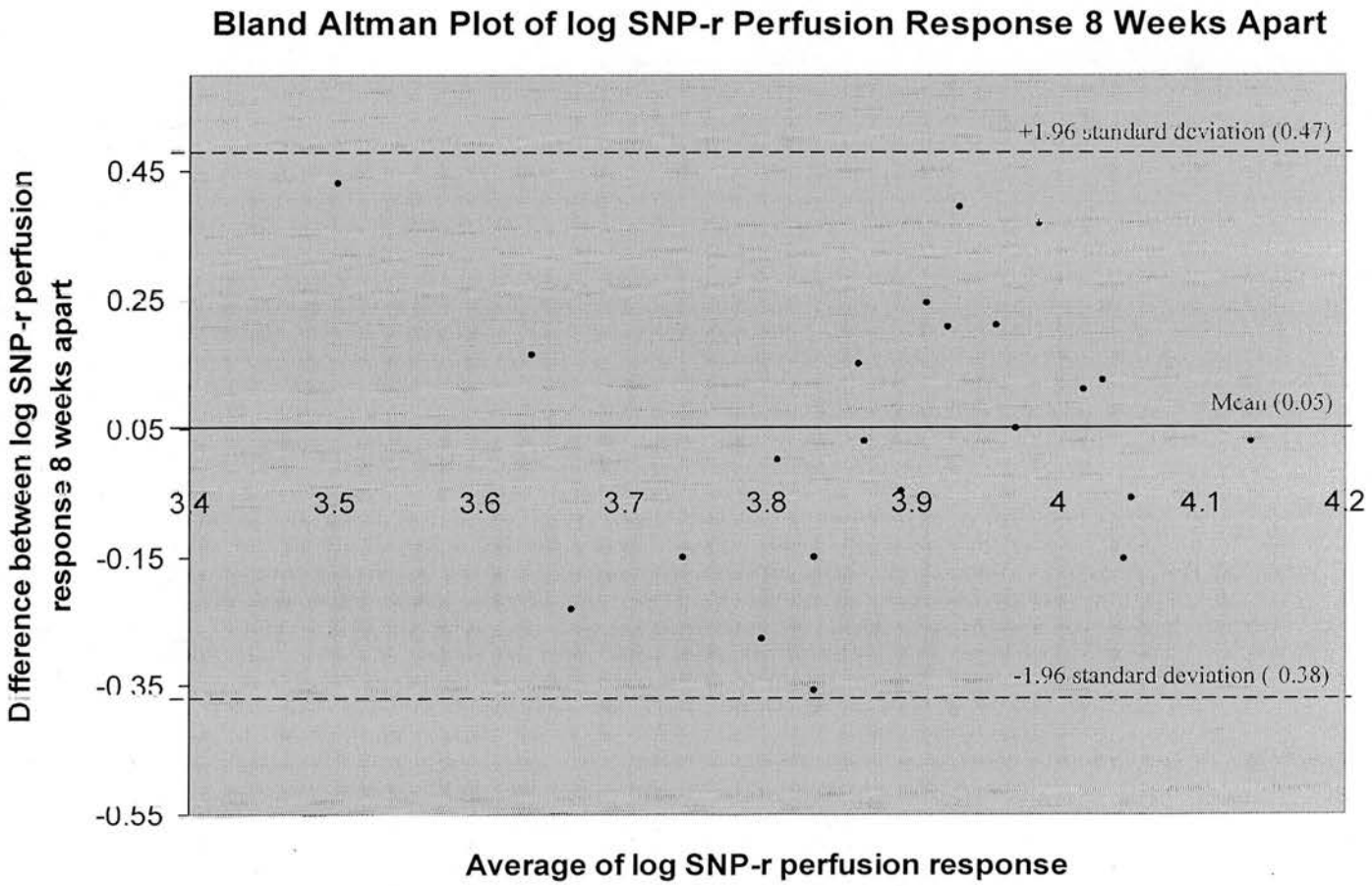
Average % diff (log)

AUC	5.9%
AUC-r	4.7%
AUC-b	15.5%
AUC-br	11.2%

Boxplot of Temporal % Difference of LDI Response to SNP




Bland Altman Plot of log SNP Perfusion Response 8 Weeks Apart
Difference between log SNP perfusion response 8 weeks apart
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
+1.96 standard deviation (0.41)
Mean (-0.01)
-1.96 standard deviation (-0.44)
2.8
2.9
3
3.1
3.2
3.3
3.4
Average of log SNP perfusion response


Bland Altman Plot of log SNP-r Perfusion Response 8 Weeks Apart
Difference between log SNP-r perfusion response 8 weeks apart
0.45
0.25
0.05
-0.15
-0.35
-0.55
+1.96 standard deviation (0.47)
Mean (0.05)
-1.96 standard deviation (0.38)
3.4
3.5
3.6
3.7
3.8
3.9
4
4.1
4.2
Average of log SNP-r perfusion response

Analysis of Temporal Variation

Boxplots above show that there is no significant difference between the measurements of LDI perfusion measurements taken 8 weeks apart without any intervention, using the unpaired t-test for both the ACh and SNP response. This, in a similar fashion to the inter-arm variation, applies to all 4 methods of analysis including the raw area under the curve value (AUC), the value adjusted for the baseline value (AUC-b), the value adjusted for the resistance-time integral (AUC-r) and the value adjusted for both (AUC-br). However, as with the inter-arm variation data, it seems that the AUC and the AUC-r values have a smaller range than AUC-b and AUC-br reflecting less variation in the in the measurements taken 8 weeks apart.

Once again, this is supported by the data looking at the mean % difference in the results 8 weeks apart. AUC and AUC-r have a much lower % difference. For the ACh response this is 4.3% and 3.5% respectively and for the SNP response it is a little higher at 5.9% and 4.7% respectively.

As before, the Bland-Altman graphs are used to look at the repeatability of the LDI measurements taken 8 weeks apart.

For the ACh AUC measurement 1 data point out of 22 (4.5%) lies outside the 95% range suggesting that the technique meets the standard for repeatability. The ACh AUC-r measurement is better, however, with only no data points lying outside the 2 standard deviations of difference. The SNP repeatability is better with no data points lying outside the 2 standard deviations for both AUC and AUC-r.

Again, it appears that the magnitude of the ACh and SNP response analysed as both the raw AUC data and the AUC-r, has no bearing on the difference seen between measurements

All of this would tend to suggest that, as with the inter-arm measurement, correction of the raw AUC data for the baseline has a detrimental effect on the repeatability of laser Doppler imaging in measuring the microvascular response to both ACh and SNP. The most robust analysis to ensure good repeatability between scan performed at different time intervals would seem to be using the raw AUC data or correcting for resistance-time integral (AUC-r). The latter appears to be slightly better than just using the raw AUC data. Better reproducibility is seen with the ACh data compared with SNP, reflected by the smaller standard deviation of the differences in the Bland-Altman plots.

Summary

There are several modes of presenting LDI perfusion data in response to iontophoresis of vasoactive drugs. The raw value obtained from the area under the curve (AUC) and the raw value corrected for skin resistance tend to give the least variability between arms and between repeat scans.

Using the log of (AUC corrected for the RTI) gives the most consistent results with an average inter-arm variability of 2.7% for ACh and 3.8% for SNP. Temporal variation is also minimal with 3.5% variability for the ACh response and 4.7% for the SNP response. Fairly good correlations between arms and between scans 8 weeks apart are also seen with the raw AUC value. Bland-Altman plots confirm that inter-arm and temporal repeat scans give reproducible data with minimal difference in mean values.

Much wider fluctuations in reproducibility are seen when the baseline values are subtracted, although there still remains no statistical difference between the data sets. The greater variability seen between scans when the baseline perfusion is subtracted may suggest that this baseline value in itself may be of some relevance. This baseline value may be a measure of vascular responsiveness or subjects starting with a high baseline value may subsequently exhibit a lower response as a result of a higher starting value. Whatever the mechanism, it seems that subtracting this baseline is not a useful exercise and the data suggest that only correction for the RTI should be made for the perfusion response.

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Ref Type: Generic

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CHAPTER 6

Examining the Difference in Microvascular Function Between Women with 'Syndrome X' at Baseline, and Healthy Controls.

Introduction

Chapter 5 dealt with the methodology behind assessment of microvascular function using iontophoresis of topically applied ACh and SNP in conjunction with laser Doppler imaging, and the reproducibility of these results.

In this chapter the differences in microvascular function between subjects recruited with cardiac 'Syndrome X' and healthy controls is examined. These data are baseline data, before any intervention. The data presented in this chapter are for both the endothelium-dependent (ACh) response and the endothelium-independent (SNP) response.

Boxplots are shown for area under the curve (AUC) data analysed by the 4 methods described in the chapter 5, although it has been shown that raw AUC and AUC corrected for resistance time integral (RTI) are the most reproducible.

Average 'dose-response' curves are also presented for response to ACh and SNP by the cardiac 'Syndrome X' group and the healthy controls. The data used in these curves are the raw values (uncorrected for RTI) but as can be seen from chapter 5, this is a good reproducible method of presenting microvascular response to ACh and SNP.

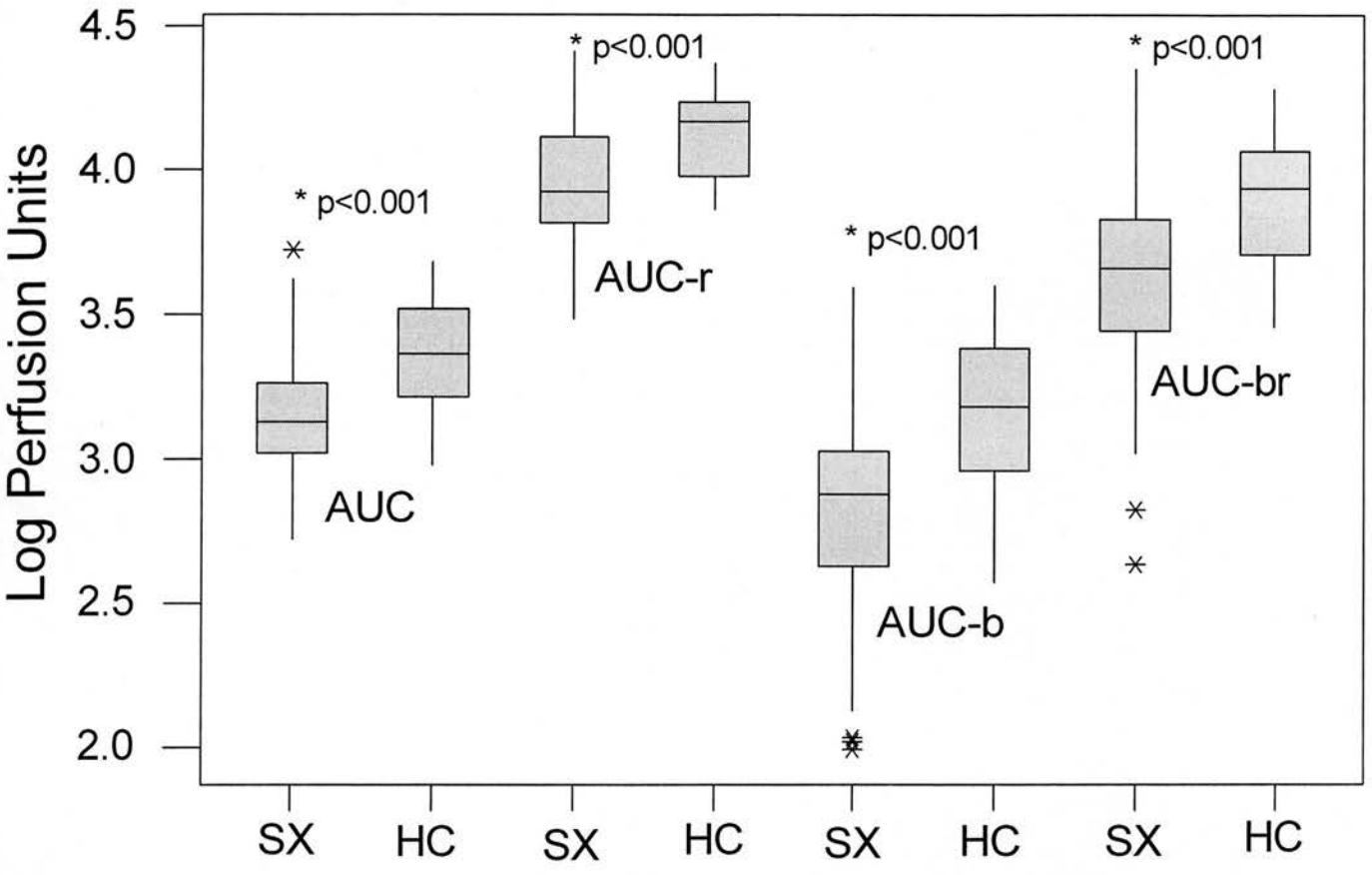
Finally in this chapter correlations are looked at between insulin resistance and microvascular function and endothelial cell markers.

1. Acetyl Choline (Endothelium-dependent) Response

The magnitude of the perfusion response to ACh is listed in the table below for the 56 'Syndrome X' patients and the 25 healthy controls (left arm data). The values have been log transformed in order to conform to normality for the purposes of box plot presentation and t-test comparison.

Patient id	ACh	ACh-r	ACh-b	ACh-br
SX001	1400	11194	850	6796
SX002	1105	15135	405	5547
SX003	5322	18008	3955	13382
SX004	2586	14264	1703	9394
SX005	961	7200	-39	-292
SX006	2430	19208	1547	12228
SX007	1128	6830	462	2797
SX008	853	5501	103	664
SX009	2032	8766	1398	6031
SX010	3243	4809	2643	3919
SX011	1388	7123	972	4988
SX012	1136	8889	319	2496
SX013	1351	6285	763	3550
SX014	1808	7503	1014	4208
SX015	1433	7833	717	3919
SX016	1265	7825	665	4114
SX017	1866	7013	1016	3818
SX018	1406	8929	896	5690
SX019	910	3444	498	1885
SX020	1647	8543	990	5135
SX021	1327	8651	720	4694
SX022	1149	7425	766	4950
SX023	1704	16668	1234	12071
SX024	1341	14179	469	4959
SX025	4180	26312	3641	22919
SX026	526	4651	134	1185
SX027	2699	20008	2121	15723
SX028	815	5467	197	1321
SX029	1087	8600	538	4256
SX030	1767	12298	924	6431
SX031	1544	8760	857	4862
SX032	776	5631	325	2358
SX033	1061	3630	355	1215
SX034	1106	4026	429	1562
SX035	1732	11331	1114	7288
SX036	864	4809	296	1648
SX037	864	5789	50	335
SX038	2462	18926	1707	13122
SX039	1848	13229	1014	7259
SX040	986	3836	496	1930
SX041	817	6364	250	1947
SX042	1359	7366	673	3634
SX043	1212	12666	673	7033
SX044	1051	10362	424	4180
SX045	1192	5850	584	2866
SX046	1611	9402	964	5626
SX047	1404	7813	825	4591
SX048	764	3078	107	431
SX049	1185	8479	577	4129
SX050	754	8167	97	1051
SX051	1533	13939	1003	9117
SX052	1024	10942	338	3612
SX053	2213	7716	1477	5150
SX054	1380	8416	752	4586
SX055	973	6568	296	1998
SX056	1881	10816	1126	6475

Control id	ACh	ACh-r	ACh-b	ACh-br
HC001	1775	8698	1010	4949
HC002	1770	14744	2405	11472
HC003	3189	17571	2621	14442
HC004	1412	8881	677	4258
HC005	1680	7375	964	4232
HC006	3445	18086	2769	14537
HC007	1078	7956	411	3033
HC008	2050	11583	1432	8091
HC009	3438	9833	2448	7001
HC010	4870	23766	4007	19554
HC011	2829	16550	2065	12080
HC012	2115	17533	1281	10619
HC013	4357	23484	3484	18779
HC014	2213	14871	1292	8682
HC015	3531	17196	2364	11513
HC016	2320	11855	1535	7844
HC017	954	7365	376	2903
HC018	3003	16727	2081	11591
HC019	1261	9470	644	4836
HC020	4467	22648	3232	16386
HC021	1506	13810	741	6795
HC022	1627	10022	872	5372
HC023	2148	15100	1256	8830
HC024	2723	14895	1968	10765
HC025	2722	15216	2055	11487



Two-Sample T-Test and CI : log ACh

Two-sample T for log ACh

group	N	Mean	StDev	SE Mean
AUC HC	25	3.368	0.193	0.039
AUC SX	56	3.146	0.190	0.025

Difference = mu (0) - mu (1)
Estimate for difference: 0.2216
95% CI for difference: (0.1287, 0.3145)
T-Test of difference = 0 (vs not =):
P-Value < 0.001

Two-Sample T-Test and CI : log ACh-r

Two-sample T for log ACh-r

group	N	Mean	StDev	SE Mean
AUC-r HC	25	4.127	0.155	0.031
AUC-r SX	56	3.945	0.208	0.028

Difference = mu (0) - mu (1)
Estimate for difference: 0.1821
95% CI for difference: (0.0989, 0.2654)
T-Test of difference = 0 (vs not =):
P-Value < 0.001

Two-Sample T-Test and CI : log ACh-b

Two-sample T for log ACh-b

group	N	Mean	StDev	SE Mean
AUC-b HC	25	3.167	0.283	0.057
AUC-b SX	55	2.830	0.355	0.048

Difference = mu (0) - mu (1)
Estimate for difference: 0.3372
95% CI for difference: (0.1890, 0.4855)
T-Test of difference = 0 (vs not =):
P-Value < 0.001

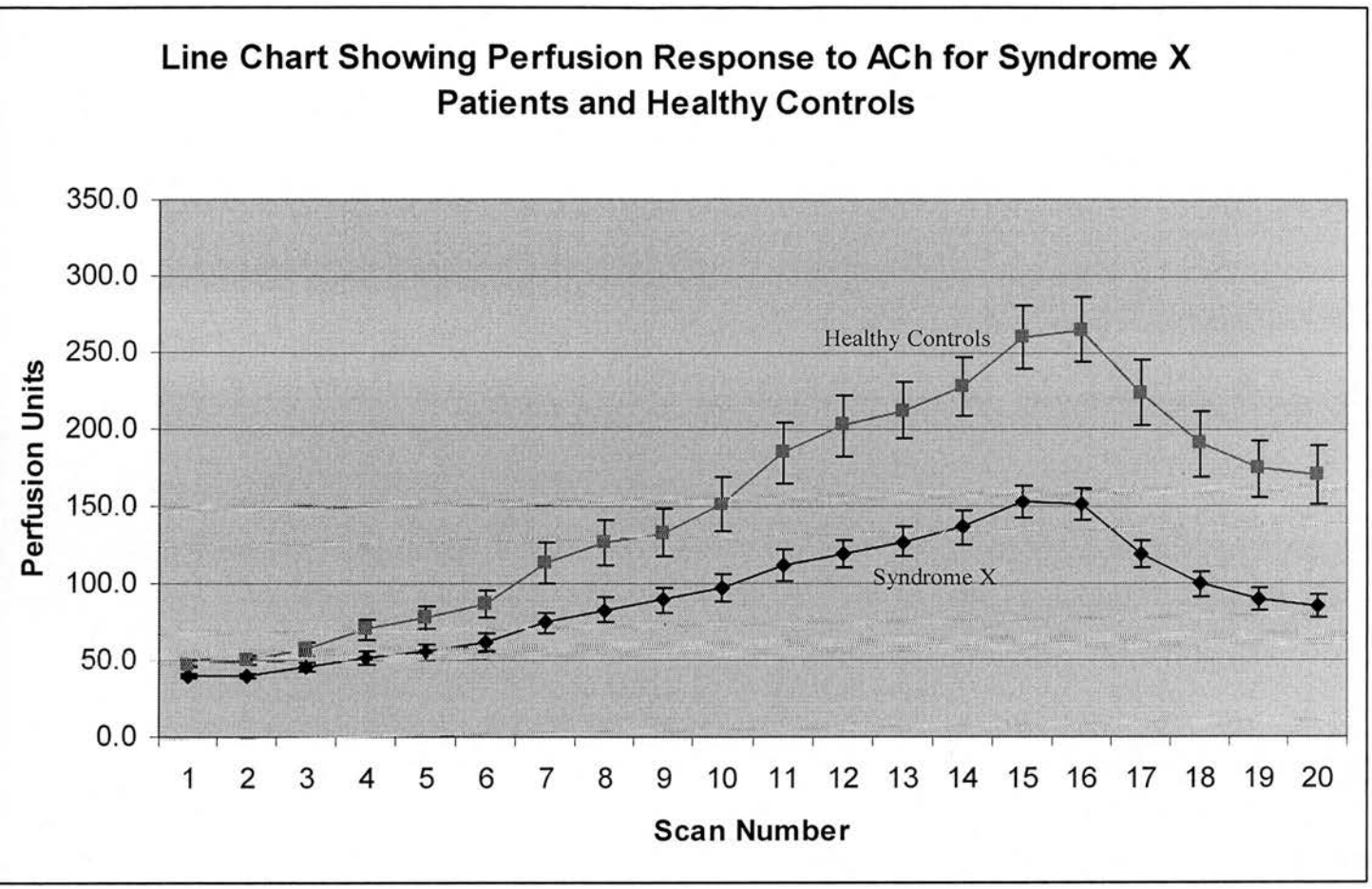
Two-Sample T-Test and C I: log ACh-br

Two-sample T for log ACh-br

group	N	Mean	StDev	SE Mean
AUC-br HC	25	3.926	0.235	0.047
AUC-br SX	55	3.627	0.343	0.046

Difference = mu (0) - mu (1)
Estimate for difference: 0.2992
95% CI for difference: (0.1676, 0.4308)
T-Test of difference = 0 (vs not =):
P-Value < 0.001

Highly significant differences in the peripheral microvascular ACh response are evident between the 'Syndrome X' group and the healthy controls with healthy controls exhibiting a significantly increased vasodilating response to ACh compared to the 'Syndrome X' group.



The above chart shows the mean perfusion response as measured by laser Doppler imaging over the 20 scans for the entire groups with cardiac 'Syndrome X' and healthy controls for ACh. As with the box charts, it can be seen that the average response differs significantly between the 2 groups. The vertical bars represent the Standard error of the mean (SEM), and the SEM for each data point do not overlap suggesting a significant difference at each data point.

These curves are analogous to a dose-response curve as the dose of ACh increases to a maximum at scan 15 with maximum current applied iontophoretically. No current is applied for the last 5 scans which is why the curves tail downwards after this point.

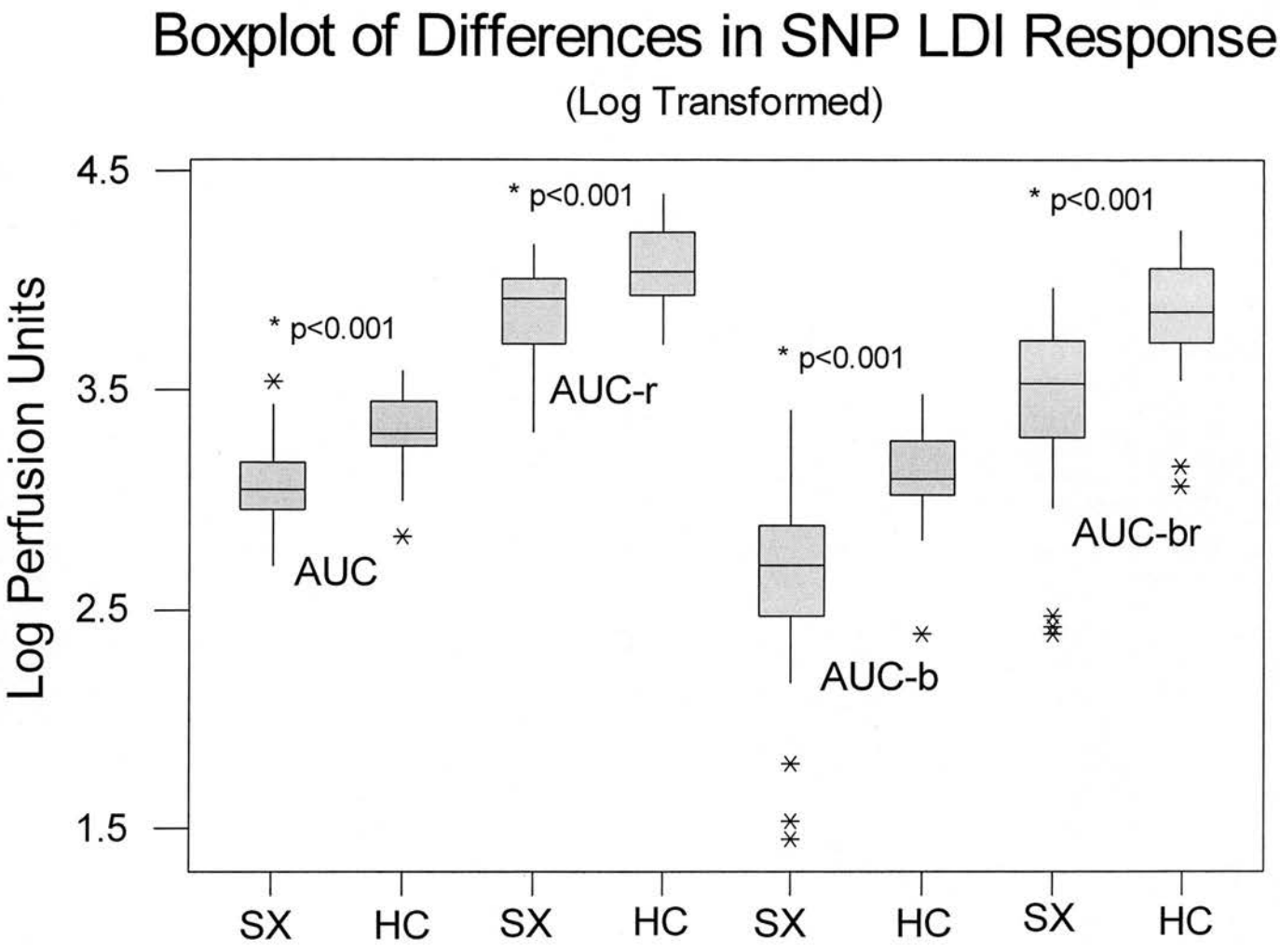
The group with cardiac 'Syndrome X' have a significantly lower vasodilating response compared to the healthy controls, suggesting impaired endothelium-dependant microvascular function.

2.Sodium Nitroprusside (Endothelium-independent) Response

The magnitude of perfusion response to SNP is listed in the table below for the 56 'Syndrome X' patients and the 25 healthy controls (left arm data). The values have again been log transformed in order to conform to normality for the purposes of boxplot presentation and t-test comparison.

Patient id	SNP	SNP-r	SNP-b	SNP-br
SX001	954	7628	471	3766
SX002	1028	14080	294	4027
SX003	3487	11799	2570	8696
SX004	1340	7391	490	2703
SX005	1228	9200	262	1963
SX006	1080	8537	347	2743
SX007	1598	9676	1114	6745
SX008	808	5211	208	1341
SX009	1019	4396	453	1954
SX010	1378	2043	678	1005
SX011	886	4547	436	2237
SX012	1482	11596	598	4679
SX013	761	3540	359	1670
SX014	2244	9312	1303	5407
SX015	1816	9927	1150	6286
SX016	872	5394	238	1472
SX017	2154	8095	1488	5592
SX018	1380	8764	959	6090
SX019	1195	4523	655	2479
SX020	1672	8673	1015	5265
SX021	1593	10385	936	6102
SX022	500	3231	166	1073
SX023	1027	10046	616	6026
SX024	1008	10658	28	296
SX025	1243	7824	704	4431
SX026	646	5712	263	2326
SX027	1674	12410	1203	8918
SX028	692	4642	152	1020
SX029	928	7342	507	4011
SX030	1322	9201	518	3605
SX031	1539	8732	598	3393
SX032	1484	10769	729	5290
SX033	1484	5077	768	2628
SX034	1260	4586	456	1660
SX035	1110	7262	473	3094
SX036	940	5232	410	2282
SX037	803	5380	146	978
SX038	1541	11846	1022	7856
SX039	1487	10645	674	4825
SX040	710	2762	63	245
SX041	574	4471	34	265
SX042	2749	14845	1729	9337
SX043	1004	10492	446	4661
SX044	1370	13507	732	7217
SX045	775	3803	187	918
SX046	1769	10324	1250	7295
SX047	884	4919	354	1970
SX048	1117	4500	587	2365
SX049	1260	9016	731	5231
SX050	793	8589	146	1581
SX051	1091	9917	502	4563
SX052	912	9745	294	3142
SX053	1896	6611	1239	4320
SX054	858	5233	201	1226
SX055	951	6419	246	1661
SX056	1298	7464	602	3462

Control id	SNP	SNP-r	SNP-b	SNP-br
HC001	2201	10785	1455	7130
HC002	1969	9392	1253	5977
HC003	3089	17020	2481	13670
HC004	1770	11133	1083	6812
HC005	1766	7753	1079	4737
HC006	2009	10547	1391	7303
HC007	1750	12915	1014	7483
HC008	1839	10390	1172	6622
HC009	2396	6853	1513	4327
HC010	3464	16904	2660	12981
HC011	991	5797	246	1439
HC012	2106	17459	1263	10470
HC013	3949	21285	3057	16477
HC014	2889	19414	1938	13023
HC015	3018	14698	1890	9204
HC016	2369	12106	1604	8196
HC017	681	5257	152	1173
HC018	2010	11196	1196	6662
HC019	1703	12790	1017	7638
HC020	2764	14013	1754	8893
HC021	2740	25126	1877	17212
HC022	1770	10903	1113	6856
HC023	3081	21659	2424	17041
HC024	1340	7330	654	3577
HC025	1414	7904	806	4506



Two-Sample T-Test and CI : log SNP

Two-sample T for log SNP raw

group	N	Mean	StDev	SE Mean
AUC HC	25	3.314	0.171	0.034
AUC SX	56	3.071	0.163	0.022

Difference = mu (0) - mu (1)
Estimate for difference: 0.2430
95% CI for difference: (0.1612, 0.3249)
T-Test of difference = 0 (vs not =):
P-Value < 0.001

Two-Sample T-Test and CI : log SNP-r

Two-sample T for log SNP-r

group	N	Mean	StDev	SE Mean
AUC-r HC	25	4.073	0.181	0.036
AUC-r SX	56	3.869	0.194	0.026

Difference = mu (0) - mu (1)
Estimate for difference: 0.2036
95% CI for difference: (0.1141, 0.2931)
T-Test of difference = 0 (vs not =):
P-Value < 0.001

Two-Sample T-Test and CI : log SNP-b

Two-sample T for log SNP-b

group	N	Mean	StDev	SE Mean
AUC-b HC	25	3.089	0.293	0.059
AUC-b SX	56	2.667	0.382	0.051

Difference = mu (0) - mu (1)
Estimate for difference: 0.4226
95% CI for difference: (0.2673, 0.5780)
T-Test of difference = 0 (vs not =):
P-Value < 0.001

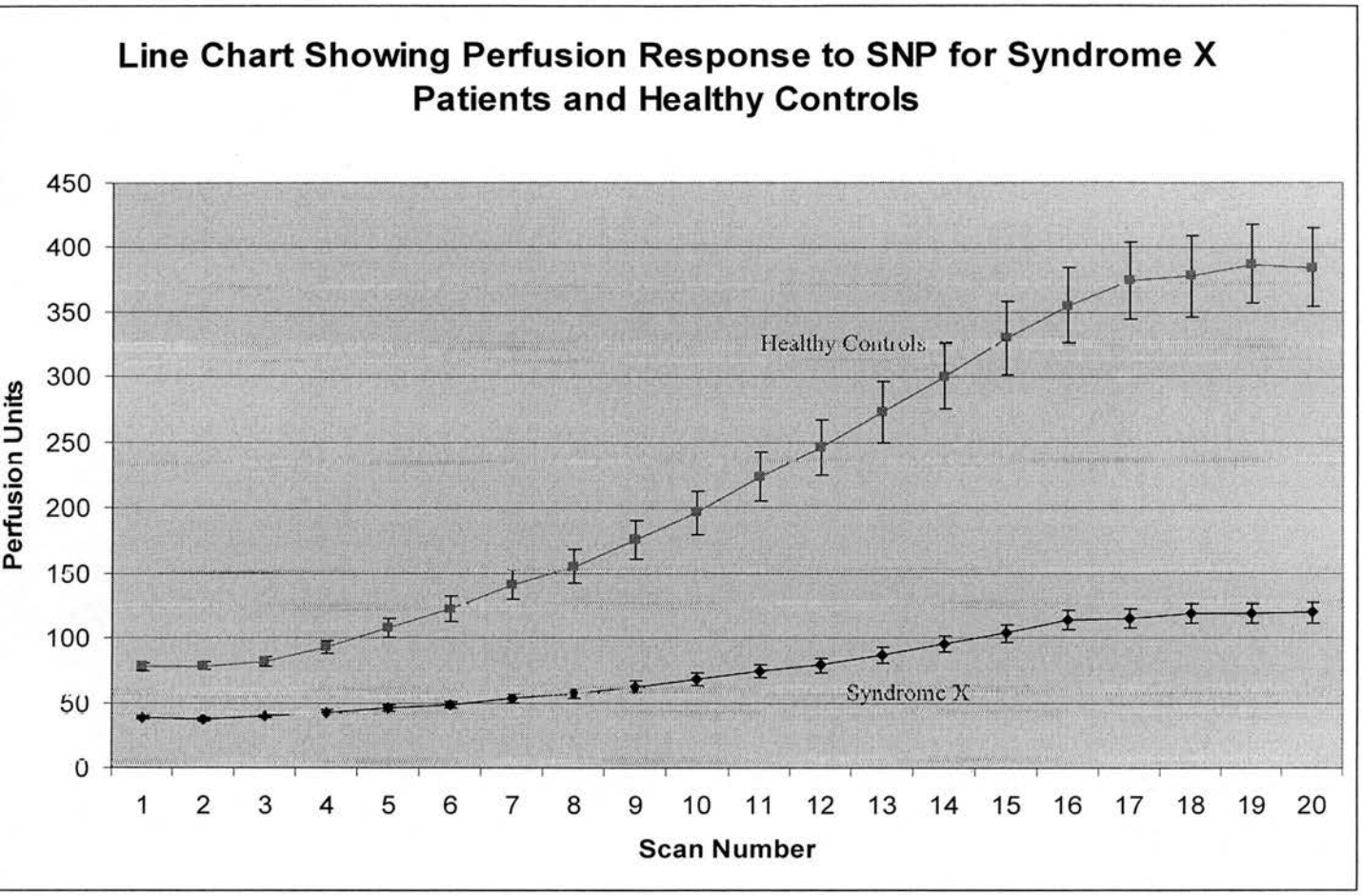
Two-Sample T-Test and CI : log SNP-br

Two-sample T for log SNP-br

group	N	Mean	StDev	SE Mean
AUC-br HC	25	3.848	0.289	0.058
AUC-br SX	56	3.465	0.365	0.049

Difference = mu (0) - mu (1)
Estimate for difference: 0.3832
95% CI for difference: (0.2317, 0.5346)
T-Test of difference = 0 (vs not =):
P-Value < 0.001

Highly significant differences in the peripheral microvascular SNP response are evident between the 'Syndrome X' group and the healthy controls with healthy controls exhibiting a significantly increased vasodilating response to ACh compared to the 'Syndrome X' group.



The above chart shows the mean perfusion response as measured by laser Doppler imaging over the 20 scans for the entire groups with cardiac 'Syndrome X' and healthy controls for SNP. As with the box charts, it can be seen that the average response differs significantly between the 2 groups. The vertical bars represent the Standard error of the mean (SEM), and the SEM for each data point do not overlap suggesting a significant difference at each data point.

These curves are analogous to a dose-response curve as the dose of SNP increases to a maximum at scan 15 with maximum current applied iontophoretically. No current is applied for the last 5 scans which is why the curves flatten out after this point. A downturn at the tail-end of the curve is not observed as for ACh, presumably due to SNP having a longer duration of action before breakdown.

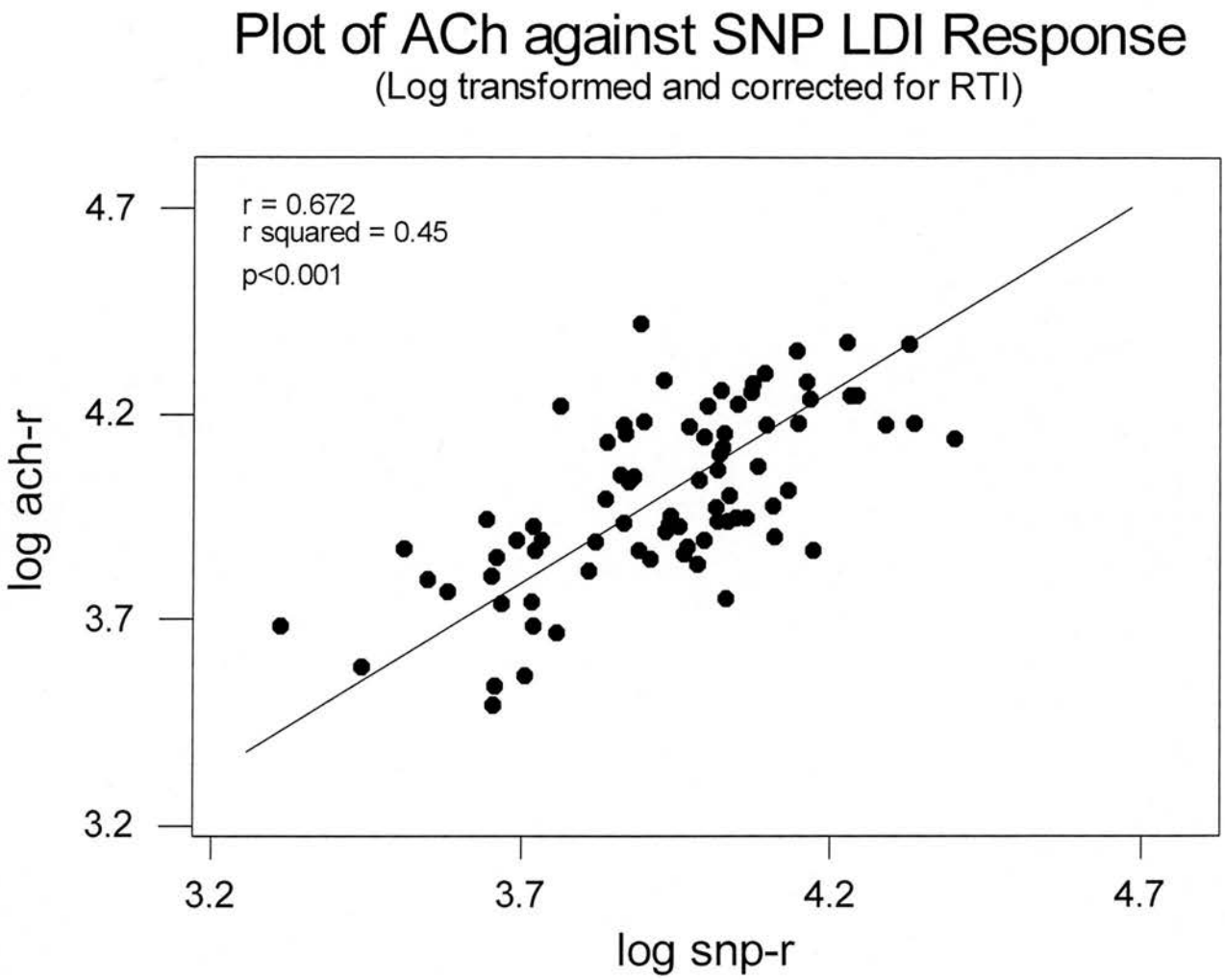
The group with cardiac 'Syndrome X' have a significantly lower vasodilating response compared to the healthy controls, suggesting impaired endothelium-independent microvascular function. This suggests that this group may have more generalised vascular smooth muscle dysfunction.

Correcting for Age and BMI

Although significant differences are seen between all of the vascular responses in the 2 groups, there are also significant differences in the ages and BMI of the 2 groups. In order to ensure that age and BMI are playing no part in the differences between the vascular responses (AUC measures) regression analysis has been employed to correct for these variables.

Variable	Difference between groups (2 sample t test)	Corrected for age	Corrected for BMI	Corrected for age and BMI (regression analysis)
ACh response	*p<0.001* *	*p<0.001**	*p<0.001* *	*p<0.001* * reg coeff = -0.21 (SE 0.05) T = -3.96
ACh response Corrected for RTI	*p<0.001* *	*p<0.001* *	*p=0.002* *	*p=0.005* * reg coeff = -0.16 (SE 0.05) T = -2.92
ACh response - baseline	*p<0.001* *	*p<0.001* *	*p<0.001* *	*p=0.001* * reg coeff = -0.32 (SE 0.09) T = -3.43
ACh response -baseline, cor RTI	*p<0.001* *	*p<0.001* *	*p=0.001* *	*p=0.003* * reg coeff = 0.27 (SE 0.09) T = -3.06
SNP response	**p<0.001** *	**p<0.001** *	**p<0.001** *	**p= 0.001** * reg coeff = 0.25 (SE 0.046) T = -5.41
SNP response Corrected for RTI	*p<0.001* *	*p<0.001* *	*p<0.001* *	*p=0.001* * reg coeff = 0.195 (SE 0.053) T = -3.7
SNP response - baseline	*p<0.001* *	*p<0.001* *	*p<0.001**	*p<0.001* * reg coeff = -0.471 (SE 0.099) T = -4.78
SNP response -baseline, cor RTI	*p<0.001* *	*p<0.001* *	*p<0.001* *	*p<0.001* * reg coeff = -0.416 (SE 0.095) T = -4.37

Even after correction for age and BMI, highly significant differences remain between the cardiac 'Syndrome X' group and the healthy controls for both endothelium-dependent and independent microvascular function.

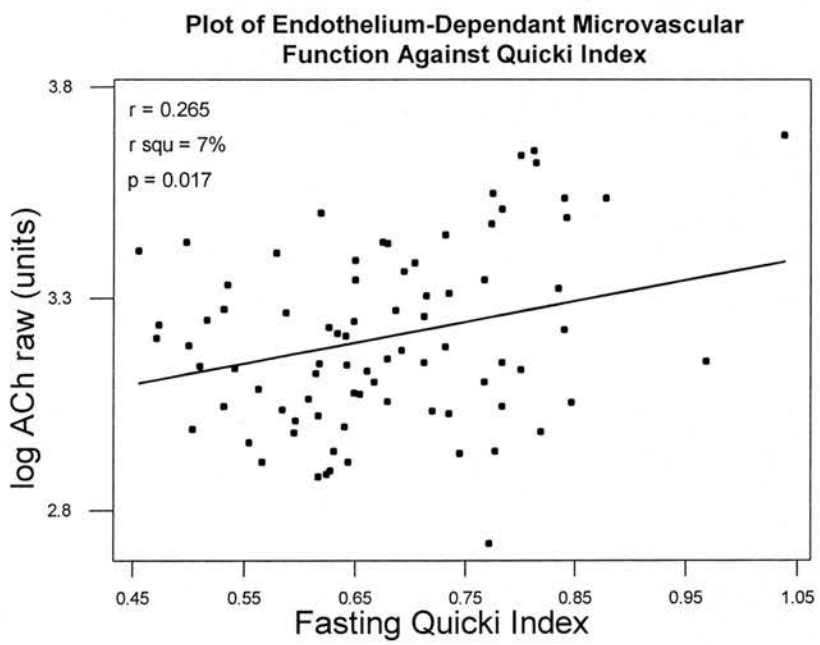


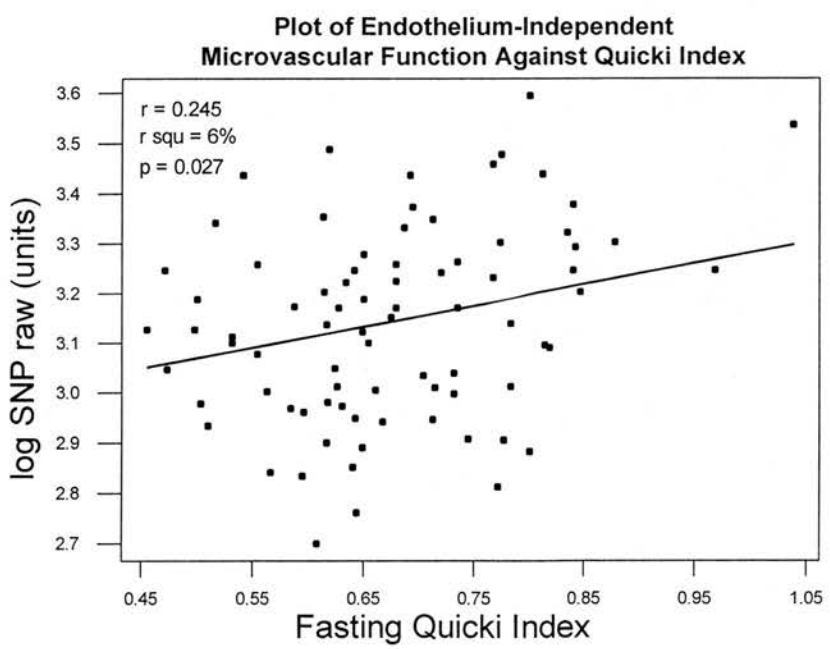
The above graph shows the relationship between the SNP and ACh response for the 'Syndrome X' patients and the healthy controls as one group. It is clear that there is a robust correlation between the endothelium-dependent ACh response and the endothelium-independent SNP response.

This would tend to suggest that the microvascular response is dependent not only on an intact endothelial monolayer but also on normally functioning vascular smooth muscle and that blunting of these two systems may occur in tandem in patients with cardiac 'Syndrome X'. It also suggests that the magnitudes of the endothelium-dependent impairment and endothelium-independent impairment are correlated.

Correlating Microvascular Function to Insulin Sensitivity

A very weak relationship exists between LDI-measured endothelium-dependant and independent microvascular function and the Quicki index of insulin sensitivity at baseline. Considering the group of healthy controls and subjects with 'Syndrome X' together ensures that the whole spectrum of measured insulin sensitivity and microvascular function are taken into account. Considering the large numbers of other variables inter-acting in a complex biological system, even a weak statistically significant association is likely to hold some relevance.

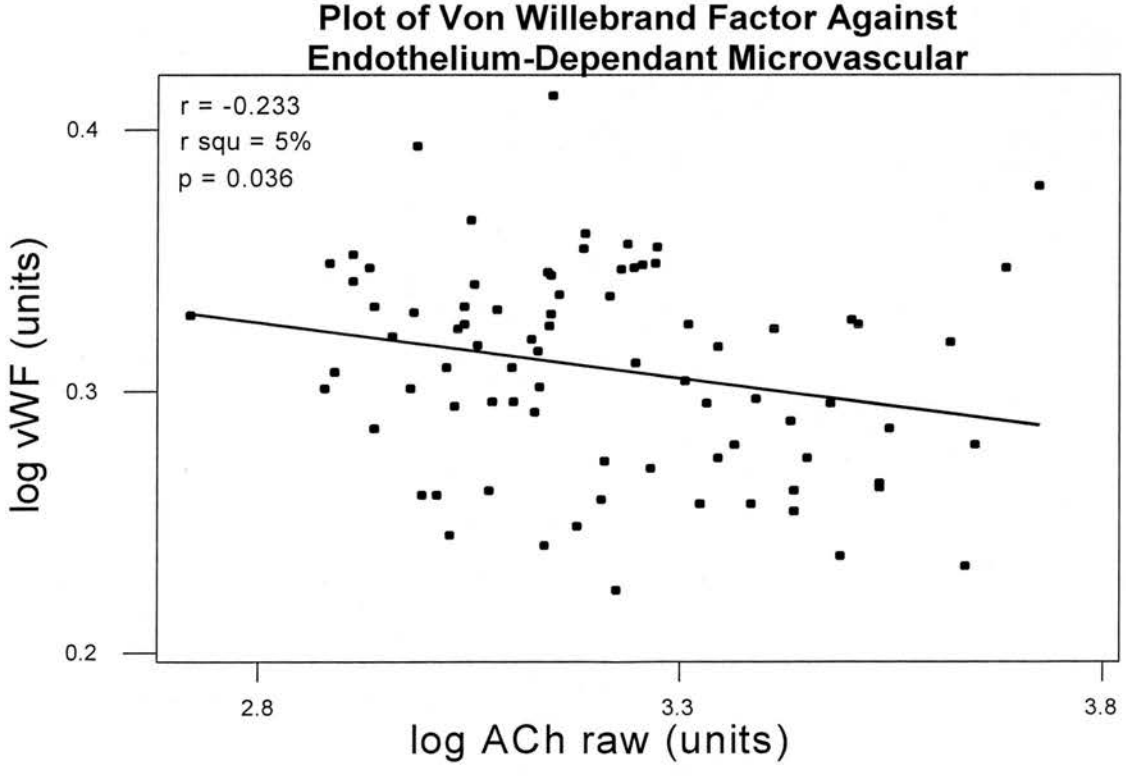




Correlating Microvascular Function to Serum Endothelial Markers

The plot below shows the relationship between the ACh perfusion response as measured by laser Doppler imaging and von Willebrand factor (a complex polypeptide produced by the endothelium and often used as a serum marker to reflect its function). The plot is for all the women at baseline ('Syndrome X' and control group) with a total of 81 data points. Again this ensures that the whole spectrum of data is considered, not just the 'Syndrome X' data, which will be at one end of the range of these variables.

A very weak but, none-the-less, significant correlation is seen between these 2 variables. This is not surprising as both are deemed to be measures of endothelial function but the correlation is weak suggesting that there are many other factors affecting these measures in-vivo. No significant association was seen with any of the other serum endothelial markers measured during the study.



Summary

There are highly significant differences between both the ACh and SNP responses of the subjects in the 2 groups and this persists even after correction for age and BMI by regression analysis. The vascular responses of the 'Syndrome X' group are attenuated compared to the controls and this suggests microvascular dysfunction at the level of the vascular smooth muscle and the endothelium (endothelium-dependent and independent responses).

There is a significant correlation between the ACh and SNP responses in all of the subjects and this suggests that those subjects with a good ACh response also have a good SNP response with the converse also being true. This may suggest a common factor being involved in the generation of both vascular responses.

Weak correlations exist between insulin sensitivity and both endothelium-dependant and independent measures of microvascular function as assessed by LDI. The r-squared values are small (7% and 6% respectively) suggesting that the interaction between these two variables is relatively small and that it is likely many other factors are at work. Interpreting this correlation data is of course difficult due to the relatively small numbers of patients and controls studied.

Although no causal role can be established from this correlation, many suggest that insulin resistance may impact upon microvascular function as discussed in chapter 2 (see figure 2 in chapter 2) A causal relationship can only be ascertained by modifying insulin resistance and looking at the effect on vascular function. Chapter 7 discusses the impact of insulin-sensitisation with metformin on microvascular function.

CHAPTER 7

Effects of Metformin on Metabolic Parameters in Women with Cardiac 'Syndrome X'

Treatment with Metformin

It has been shown that women with 'Syndrome X' exhibit insulin resistance compared to healthy controls as well as several features of the metabolic syndrome and impaired peripheral microvascular function. The rationale for this double-blinded placebo-controlled trial was to determine whether metformin, as an insulin-sensitising modality, would have any effect on:

- Indices of insulin resistance
- Other features of the metabolic syndrome
- Serum markers of endothelial function.
- Anthropometric data

Biguanides

This class of drug has been used for almost 50 years in clinical practice for the treatment of type 2 diabetes. They reduce hyperglycaemia without causing weight gain or hypoglycaemia. Metformin is widely prescribed and phenformin is still used in some countries, although it has a higher association with lactic acidosis.

Metformin has been looked at mainly in the context of type 2 diabetes where its principal effect is on hepatic insulin sensitivity. It reduces hepatic glucose output by a reduction in hepatic gluconeogenesis with a possible effect on glycogenolysis also (1). There is also evidence that metformin increases hepatic glucose uptake and glycogen synthesis, via a pathway involving glucose-derived 3-carbon units. Less significant end-products of these 3-carbon units include lactate and pyruvate, both of which are seen to increase in subjects treated with metformin (1;2).

Metformin also has an effect on peripheral tissue insulin-sensitivity, primarily in skeletal muscle. In vitro studies of rat muscle have shown that samples treated with metformin have an increased rate of insulin-mediated glucose uptake and glycogensis (3). There is also evidence to imply that this effect extends to human tissues - muscle samples obtained both from insulin-resistant subjects and grown from cell-culture, show improved insulin-mediated glucose uptake when treated with metformin (3).

There is emerging evidence that metformin may influence the secretory function of islet cells directly, from rat models. Rat islet cells whose insulin-secretory capacity had been impaired by long-term exposure to elevated free fatty acid and glucose concentrations in vitro, showed improvements in normal secretory function when treated with metformin (4). Whether or not these data will have any relevance to human physiology is yet to be determined.

As well as direct effects on glucose metabolism, metformin also has appreciable effects on other features of the insulin resistance syndrome. The BIGPRO trial was a prospective study designed to look at these parameters and included 324 non-diabetic individuals with central obesity (5;6). Significant reductions in fasting insulin levels were seen after one year of metformin compared with placebo as would be expected. However, significant weight loss also occurred together with a reduction in total and LDL-cholesterol. The fibrinolytic system also showed differences with a reduction in the level of tissue-type plasminogen activator antigen, although plasminogen activator inhibitor levels were not significantly changed. No significant effect was seen on blood pressure or plasma triglyceride levels.

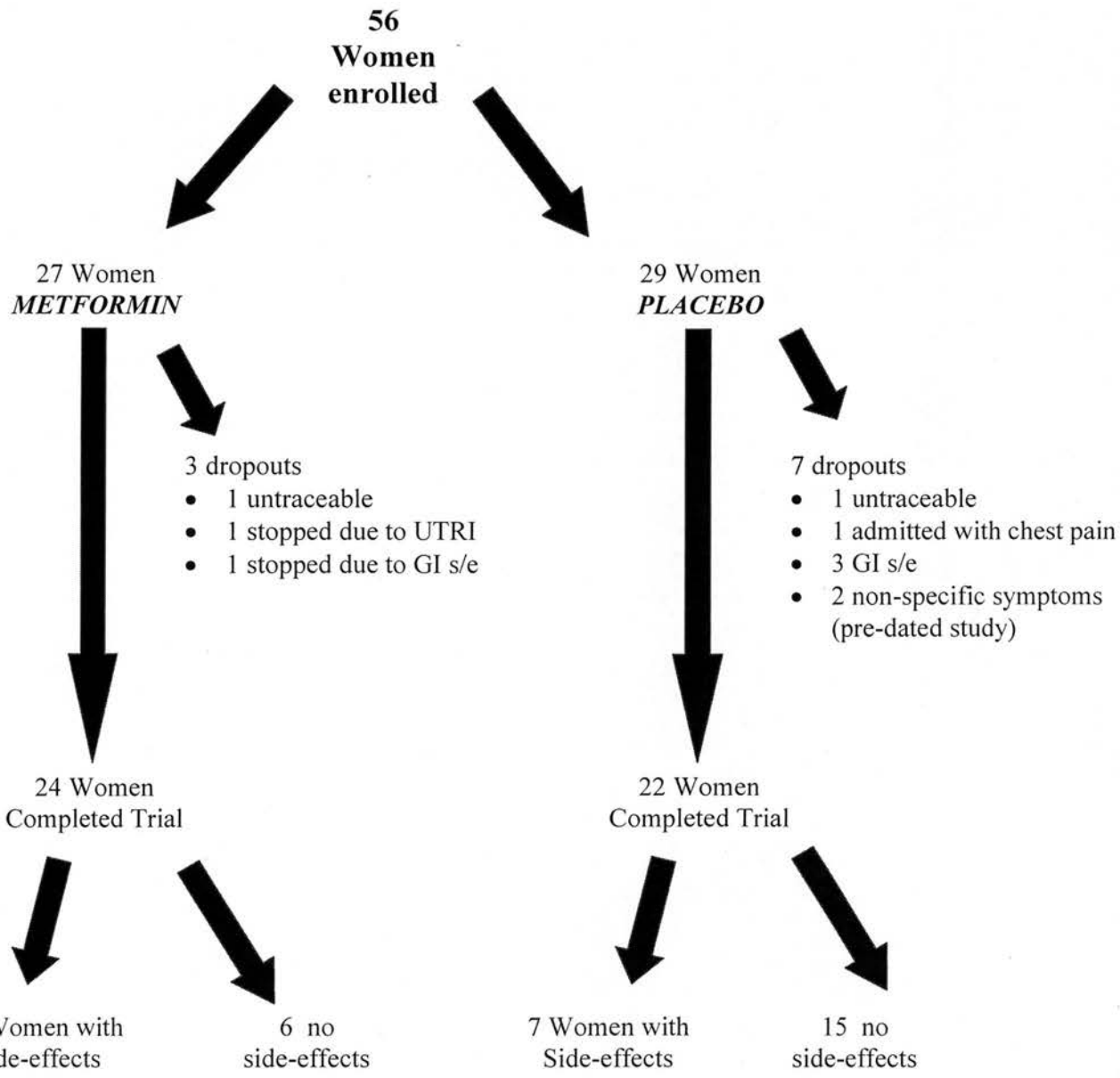
A similar trial in subjects with type 2 diabetes did show a significant reduction in triglyceride levels and plasminogen activator inhibitor-1 after 12 weeks of metformin treatment compared with 12 weeks of placebo in a cross-over fashion. As with the BIGPRO data, blood pressure was unaffected (7).

Clear benefits were seen with metformin in the UK Prospective Diabetes Study (UKPDS). Overweight patients with type 2 diabetes treated with metformin, showed a clear reduction in all cause mortality (36%), myocardial infarction (39%) and total macrovascular disease (30%) encompassing stoke, angina, myocardial infarction, sudden death and peripheral vascular disease, compared to dietary treatment alone. More importantly, when compared to patients assigned to intensive therapy with sulphonylureas or insulin, the metformin group had a significantly greater reduction in all-cause mortality. Smaller reductions in the incidence of myocardial infarction and stroke were seen in the metformin group, compared with the sulphonylureas/insulin group, but these reductions were not statistically significant (8).

This observation would suggest that the benefit in all-cause mortality seen with metformin can not in full be attributed to better glycaemic control alone. In fact, median glycated haemoglobin levels were similar between the group assigned metformin and the group assigned sulphonylureas or insulin. The improvements in insulin sensitivity and the other features of the insulin resistance syndrome are the likely mediators of this benefit.

Tolerability and Side-effects of Metformin

56 women were enrolled in the MIRS Study initially. 27 women were randomised to metformin and 29 received placebo. The flowchart below outlines the incidence and nature of the side-effects encountered.



- 14 with mild short-lived GI upset
- 1 with more prolonged GI upset
- 3 with other side-effects

- All 7 with mild transient GI upset

Serum Lactate

Lactic acidosis has always been a concern during treatment of patients with metformin, especially high risk groups with renal impairment or cardiac failure. I measured serum lactate before and after treatment in the 'Syndrome X' cohort as well as in the healthy controls. Log transformation of the raw data was required to establish a normal distribution. Results are shown below in table form and boxplots. In one subject in the Syndrome X group no serum sample was obtained.

Syndrome X at baseline

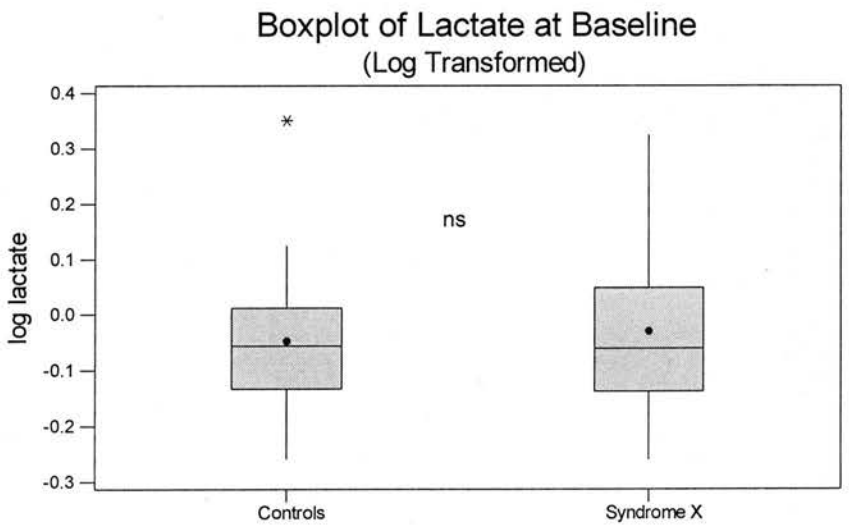
Patient ID	Serum lactate (mmol/L)
SX001	0.71
SX002	0.68
SX003	0.86
SX004	0.86
SX005	1.01
SX006	0.75
SX007	0.69
SX008	0.85
SX009	0.55
SX010	1.23
SX011	0.74
SX012	0.73
SX013	0.94
SX014	0.81
SX015	0.87
SX016	1.03
SX017	0.73
SX018	0.95
SX019	0.98
SX020	0.81
SX021	0.85
SX022	1.07
SX023	0.76
SX024	1.35
SX025	0.73
SX026	0.62
SX027	0.69
SX028	1.03
SX029	0.87
SX030	0.90
SX031	0.72
SX032	1.11
SX033	1.03
SX034	1.01
SX035	0.86
SX036	0.70
SX037	0.65
SX038	1.19
SX039	1.29
SX040	0.76
SX041	1.22
SX042	1.35
SX043	0.63
SX044	1.14
SX045	1.30
SX046	0.66
SX047	1.60
SX048	0.74
SX049	1.00
SX050	1.12
SX051	2.11
SX052	1.39
SX053	1.93
SX054	0.95
SX055	1.62

Healthy Controls

Control ID	Serum lactate (mmol/L)
HC001	2.24
HC002	0.68
HC003	0.88
HC004	0.90
HC005	0.92
HC006	0.73
HC007	0.80
HC008	0.74
HC009	0.67
HC010	0.78
HC011	0.55
HC012	0.74
HC013	1.03
HC014	1.29
HC015	0.77
HC016	0.72
HC017	0.72
HC018	0.89
HC019	0.84
HC020	0.91
HC021	1.03
HC022	1.03
HC023	0.94
HC024	1.33
HC025	1.32

Syndrome X (Metformin)	
Serum lactate (mmol/L) Baseline	**Serum lactate (mmol/L) After 8 weeks**
0.86	1.06
1.01	1.23
0.69	0.65
0.55	0.96
1.23	1.96
1.03	1.68
0.73	0.91
0.95	1.06
0.98	1.08
0.85	0.78
0.73	0.79
0.87	1.37
0.90	0.89
1.11	0.67
1.03	1.35
1.19	1.23
1.29	2.19
0.76	0.84
1.14	1.29
0.66	0.75
0.74	0.66
1.00	0.92
2.11	1.57

Syndrome X (Placebo)	
Serum lactate (mmol/L) Baseline	**Serum lactate (mmol/L) After 8 weeks**
0.71	1.24
0.68	0.65
0.86	0.95
0.74	0.73
0.73	0.96
0.94	0.93
0.81	1.38
0.81	0.83
1.35	2.63
0.62	0.68
0.69	1.11
1.03	0.88
0.72	0.83
0.86	0.9
0.65	0.79
1.22	1.22
1.35	1.23
0.63	0.64
1.60	1.22
1.12	1.61
1.93	1.65
0.95	0.89



Two-Sample T-Test and CI : log Lactate at Baseline

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Two-sample T for log lactate (Syndrome X Vs Controls)

group           N      Mean     StDev   SE Mean
Controls       25    -0.048     0.126     0.025
Syndrome X     55    -0.030     0.128     0.017

Difference = mu (0) - mu (1)
Estimate for difference:  -0.0178
95% CI for difference: (-0.0791, 0.0435)
T-Test of difference = 0 (vs not =):

P-Value = 0.563
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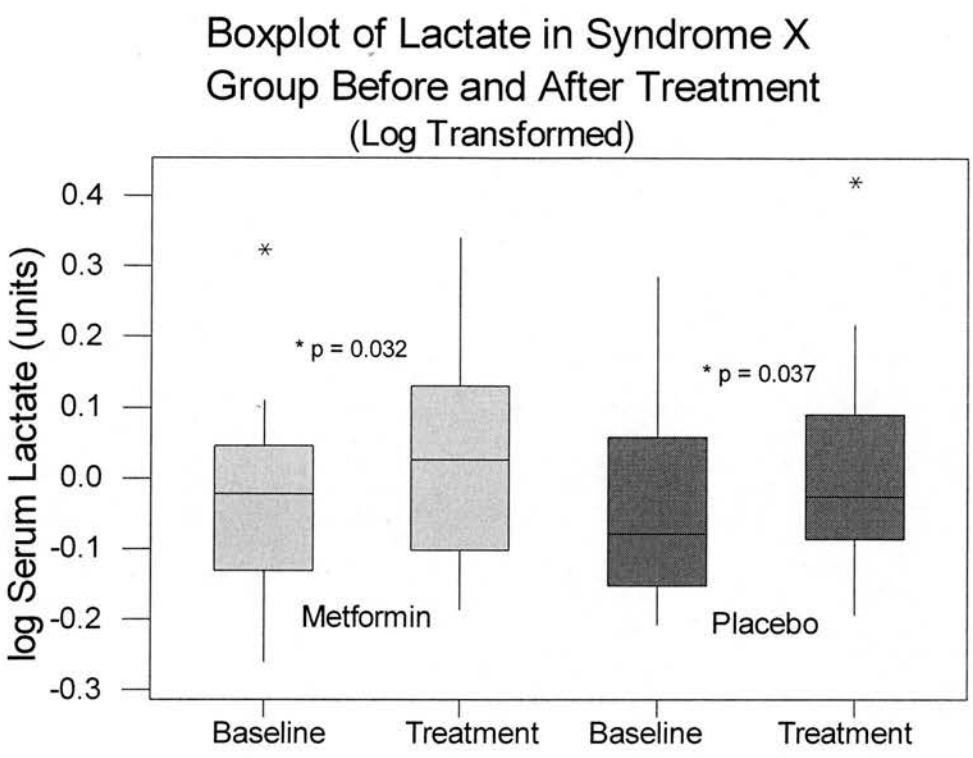


Paired T-Test and CI : log lactate (Metformin)

Paired T for log lactate before and after metformin

	N	Mean	StDev	SE Mean
Baseline	23	-0.0289	0.1221	0.0255
Metformin	23	0.0260	0.1490	0.0311
Difference	23	-0.0549	0.1147	0.0239

95% CI for mean difference: (-0.1045, -0.0053)
T-Test of mean difference = 0 (vs not = 0):

P-Value = 0.032

Paired T-Test and CI: log lactate (Placebo)

Paired T for log lactate before and after placebo

	N	Mean	StDev	SE Mean
Baseline	22	-0.0431	0.1386	0.0296
Placebo	22	0.0097	0.1496	0.0319
Difference	22	-0.0527	0.1108	0.0236

95% CI for mean difference: (-0.1018, -0.0036)
T-Test of mean difference = 0 (vs not = 0):

P-Value = 0.037

Analysis of Lactate Data

75% of women treated with metformin had side-effects on treatment with the vast majority being mild transient gastro-intestinal (GI) upset. This compares with 32% on placebo complaining of gastro-intestinal side-effects. Only 1 woman who completed the study had more prolonged and sustained GI side-effects with metformin.
Despite this higher rate of side-effects only 3 dropouts were seen in the metformin arm (13%) as compared to 7 women (32%) with placebo.

Serum lactate was not significantly different between the 'Syndrome X' group and the controls at baseline. After 8 weeks both the 'Syndrome X 'group treated with metformin and the group treated with placebo, showed a very small increase in serum lactate.
The average lactate level rose from 0.97 mmol/L to 1.13 mmol/L in the metformin group (an average rise of 17%). This is a similar increment to that seen in the placebo-treated group (0.95 mmol/L to 1.09 mmol/L – an average rise of 17% also)

In summary, although the incidence of GI side-effects was relatively high with metformin (75%), in most cases it took the form of a very mild transient GI upset. Overall, metformin was well-tolerated with fewer dropouts than seen with placebo. Although a minor increase in serum lactate was seen during metformin treatment, it was statistically significant. However, a comparable rise was observed in the placebo group. The cause for this increase in the placebo group is not clear but it does suggest that the small increase in lactate observed during treatment with metformin may not be related to metformin treatment per se. Lactic acidosis was not a significant problem.

Baseline Characteristics

Table 7.1 illustrates the baseline characteristics of the 2 groups of women with cardiac 'Syndrome X'. There were similar numbers of smokers and postmenopausal women in both groups. Rates of drug-use were similar between the groups with the exception that slightly more women in the metformin-treated group took statins and calcium-channel blockers for the duration of the study. The difference for calcium channel blockers just reached statistical significance.

As can be seen from table 7.2, the metformin and placebo groups of women with cardiac 'Syndrome X' were well matched at baseline in terms of most of the clinical, metabolic, anthropometric and vascular variables measured. The only statistically significant difference seen between the groups was with respect to GTN usage. The placebo-treated group used less GTN than the metformin-treated group at baseline and this may therefore make any conclusions about the change in GTN usage following treatment difficult to interpret.

Table7. 1 Baseline characteristics of study population in terms of smoking, menopause and drug use

	Metformin group n=24	***Placebo group*** n=22	**p value***
Smoker	8	9	*ns*
Postmenopausal	17	14	*ns*
HRT use	7	7	*ns*
Aspirin	18	16	*ns*
Statin use	13	7	*ns*
Beta blockers	0	1	*ns*
Nitrate	3	2	*ns*
Calcium channel blockers	4	0	*0.045*
Other antihypertensive	2	2	*ns*
ACE inhibitor	2	3	*ns*
Angiotensin receptor blocker	1	0	*ns*

* p value calculated by 2x2 chi-squared test

Characteristics of Metformin-treated Group and Placebo-treated Group

Table 7.2. Clinical characteristics of the metformin and placebo group at baseline. † mean +/- standard deviation. ‡ geometric mean +/- standard deviation

	Metformin n=24	Placebo n=22	P value
Age	56.6 +/- 7.83 †	57.0 +/- 6.98 †	NS
Systolic blood pressure (mmHg)	131.3 +/- 19.5†	134.9 +/- 15.7†	NS
Diastolic blood pressure (mmHg)	79.5 +/- 8.4†	80.1 +/- 9.8†	NS
Total Cholesterol (mmol/L)	4.92 +/- 0.92†	4.92 +/- 1.05†	NS
Triglycerides (mmol/L)	1.32 +/- 1.42‡	1.47 +/- 1.95‡	NS
LDL-cholesterol (mmol/L)	3.03 +/- 0.71	2.98 +/- 0.89	NS
HDL-cholesterol (mmol/L)	1.40 +/- 0.36	1.31 +/- 0.33	NS
Fasting Glucose (mmol/L)	4.88 +/- 0.42	4.87 +/- 0.36	NS
Fasting Insulin	7.00 +/- 1.61‡	8.26 +/- 1.71‡	NS
Fasting Quicki index	0.67 +/- 0.098	0.64 +/- 0.091	NS
Von Willebrand Factor	119.95 +/- 1.49‡	130.32 +/- 1.40‡	NS
Tissue plasminogen activator	7.40 +/- 2.96	8.69 +/- 2.62	NS
D-Dimer	81.3 +/- 1.89‡	78.3 +/- 1.92‡	NS
I-CAM	256.4 +/- 1.27‡	235.0 +/- 1.25‡	NS
V-CAM	345.1 +/- 1.27‡	319.3 +/- 1.25‡	NS
Waist (cm)	85.5 +/- 7.9	90.0 +/- 12.1	NS
Waist : Hip ratio	0.79 +/- 0.043	0.81 +/- 0.058	NS
Weight (kg)	71.7 +/- 9.65	74.0 +/- 10.5	NS
Body Mass Index (kg/m^2)	28.3 +/- 4.08	29.2 +/- 4.99	NS
Leptin	35.7 +/- 12.9	38.8 +/- 12.5	NS
C-reactive protein	2.85 +/- 1.97‡	3.17 +/- 4.02‡	NS

Presentation of Results

The results for the measured metabolic, clinical and anthropometric variables before and after metformin and placebo are presented in the following pages.
The raw data are shown in a table for both the metformin and placebo groups. Data presented are on the women who completed the trial follow-up, therefore data on 24 women in the metformin group and 22 in the placebo group are shown. Some samples were unsuitable for analysis which leads to missing data points in some tables.

Subsequently, the outcomes are first shown in tabular form with the mean and standard error of mean (SEM) shown at baseline and after the 8 weeks of intervention with either metformin or placebo. The statistical significance of the change is shown next to each set of measurements. Statistical non-significance is represented by 'ns' whereas the p valve for any significant change is shown.

Additionally, the change for each variable, before and after intervention, was calculated for all of the women in both groups, and the difference between this change in both groups was looked at. The difference was compared statistically between the groups, using a 2-sample t-test, and this result is shown in the last column of each table. Again 'ns' is simply used to imply that the difference in the mean change between the groups is not statistically significant, with a p valve being presented only when the difference achieved statistical significance.

Finally a boxplot is shown for the important variables measured with the central bar representing the mean and the box representing the standard deviation of the data set. Data are presented as a boxplot for each time point on the oral glucose tolerance test for the insulin/glucose metabolism data in section 1. Metformin-treated subjects are displayed adjacent to their placebo-treated counterparts. This is shown to more graphically illustrate the change in variables measured before and after intervention.

1 : Effects of Metformin on Indices of Glucose/Insulin Metabolism

The raw data are shown in the tables below for the metformin and placebo groups at baseline and then post intervention.

Metformin Group at Baseline

gluc 0 mmol/l	gluc 60 mmol/l	gluc 120 mmol/l	ins 0 mu/l	ins 60 mu/l	ins 120 mu/l	quicki 0 units	quicki 60 units	quicki 120 units	NEFA 0 mmol/l	NEFA 60 mmol/l	NEFA 120 mmol/l
5.2	11.6	9.2	15.2	90.5	107.2	0.53	0.33	0.33	0.77	0.3	0.09
4.5	10.2	8.7	3.7	63.4	47.2	0.82	0.36	0.38	0.8	0.24	0.1
4.6	3.6	5.9	3.3	20.7	33.9	0.85	0.53	0.43	0.45	0.05	0.05
4.4	8.5		5.7	108.2	70.6	0.71	0.34		0.84	0.04	0.02
4.4	9.4	7.8	4.3	116.6	68.9	0.78	0.33	0.37	0.72	0.13	0.12
4.5	7.6	6.7	7.0	73.3	39.8	0.67	0.36	0.41	0.24	0.04	0.03
5.3	14.2		5.4	65.3	32.8	0.69	0.34		0.66	0.07	0.05
4.2	6.7	5.7	4.5	36.9	32.9	0.78	0.42	0.44	0.67	0.22	0.02
4.5	6.4	9.6	14.2	90.7	185.4	0.55	0.36	0.31	0.8	0.16	0.06
4.6	9.5	6.9	9.2	139.7	82.9	0.61	0.32	0.36	0.58	0.08	0.04
4.7	7.7	5.7	3.6	83.1	47.6	0.81	0.36	0.41	0.57	0.07	0.03
4.9	7.9	4.5	7.1	71.4	42.8	0.65	0.36	0.44	0.43	0.06	0.05
5.5	13.9	8.4	18.2	169.5	106.8	0.50	0.30	0.34	0.59	0.07	0.03
5.0	10.4	5.1	4.6	95.7	26.8	0.73	0.33	0.47	0.42	0.12	0.05
5.5	18.6	14.1	13.9	198	289.7	0.53	0.28	0.28	0.56	0.26	0.07
5.6	11.3	7.8	9.0	29.6	32.6	0.59	0.40	0.42	0.52	0.17	0.05
5.6	6.7	7.2	6.5	37.4	51	0.64	0.42	0.39	0.47	0.14	0.07
5.2	5.3	6.1	6.9	59.7	47.4	0.64	0.40	0.41	0.4	0.11	0.03
4.7	9	6.5	7.4	100.5	56.8	0.65	0.34	0.39	0.34	0.11	0.04
4.7	6.7	6.5	5.4	50.5	65.7	0.71	0.40	0.38	0.41	0.04	0.02
4.7	6.7	5.8	7.2	64.9	45.3	0.65	0.38	0.41	0.51	0.04	0.03
5.1	7.4	5.5	8.2	172.4	109.1	0.62	0.32	0.36	0.35	0.13	0.08
4.9	7.1	8.7	9.7	147.8	97.3	0.60	0.33	0.34	0.57	0.09	0.05

Metformin Group post intervention

gluc 0 mmol/l	gluc 60 mmol/l	gluc 120 mmol/l	ins 0 mu/l	ins 60 mu/l	ins 120 mu/l	quicki 0 units	quicki 60 units	quicki 120 units	NEFA 0 mmol/l	NEFA 60 mmol/l	NEFA 120 mmol/l
5.7	15.2	11.5	15.7	107.6	65.1	0.51	0.31	0.35	0.76	0.35	0.05
4.6	11.8	9.6	3.7	71	56.7	0.81	0.34	0.37	0.77	0.18	0.05
4.4	7.9	4.8	2.9	68.3	22.5	0.90	0.37	0.49	0.59	0.08	0.02
4.7	7.1	7.2	0.7	44.5	38.7	1.93	0.40	0.41	0.75	0.18	0.06
4.3	9.9	8.0	4.0	125.8	96.1	0.81	0.32	0.35	0.76	0.15	0.12
4.5	5.2	5.1	8.8	55.9	28.5	0.63	0.41	0.46	0.32	0.12	0.14
5.1	14.1	14.4	5.7	36.6	40.5	0.68	0.37	0.36	1.01	0.16	0.06
4.9	8.6		5.0	30.9		0.72	0.41		0.79	0.47	
4.4	8.6	7.0	7.1	49.2	90.4	0.67	0.38	0.36	0.58	0.08	0.04
4.5	12.0	8.0	6.6	117.5	64.1	0.68	0.32	0.37	0.69	0.08	0.05
4.6	9.8	7.4	5.1	70.2	57.2	0.73	0.35	0.38	0.72	0.19	0.04
4.8	7.5	4.2	5.9	65.4	35.3	0.69	0.37	0.46	0.34	0.12	0.06
4.7	8.6	9.1	7.3	51.2	49.8	0.65	0.38	0.38	0.63	0.32	0.09
5.7	10.7	2.9	3.3	106	7.8	0.78	0.33	0.74	0.33	0.06	0.04
4.6	15.2	11.0	13.0	142.3	165.4	0.56	0.30	0.31	0.55	0.28	0.09
4.9	9.9	10.0	6.6	21.9	42	0.66	0.43	0.38	0.62	0.18	0.07
5.0	6.5	6.4	6.8	14.4	33.6	0.65	0.51	0.43	0.35	0.12	0.08
5.1	6.0	5.8	10.9	97.1	52.3	0.57	0.36	0.40	0.14	0.08	0.05
4.7	9.6	8.5	7.7	64.5	81.7	0.64	0.36	0.35	0.39	0.15	0.04
4.5	6.4	6.8	6.1	66.5	58.5	0.70	0.38	0.38	0.47	0.09	0.03
4.7	6.5	6.4	6	41.4	45.2	0.69	0.41	0.41	0.46	0.04	0.02
5.2	8.5	4.7	7.4	423.6	94.7	0.63	0.28	0.38	0.39	0.11	0.07
5.1	8.0	6.8	10.1	92.5	72.2	0.58	0.35	0.37	0.56	0.14	0.08

Placebo Group at Baseline

gluc 0 mmol/l	gluc 60 mmol/l	gluc 120 mmol/l	ins 0 mu/l	ins 60 mu/l	ins 120 mu/l	quicki 0 units	quicki 60 units	quicki 120 units	NEFA 0 mmol/l	NEFA 60 mmol/l	NEFA 120 mmol/l
5.0	13.3	9.0	8.3	79.0	153.3	0.62	0.33	2.19	0.55	0.08	0.03
4.4	8.4	8.2	4.3	54.9	57.4	0.78	0.38	1.76	0.46	0.08	0.06
4.7	11.8	7.2	33.6	1005.6	562.8	0.45	0.25	2.75	0.67	0.09	0.07
4.4	10.5	8.2	8.2	112.1	182.0	0.64	0.33	2.26	0.7	0.23	0.04
5.3	7.3	7.2	5.6	76.4	50.0	0.68	0.36	1.70	0.54	0.05	0.04
4.8	10.2	8.6	3.7	45.2	45.5	0.80	0.38	1.66	0.89	0.17	0.07
4.6	9.4	5.2	5.5	63.3	16.4	0.71	0.36	1.21	0.84	0.09	0.05
5.4	12.1	8.9	7.0	96.7	93.5	0.63	0.33	1.97	0.72	0.21	0.04
4.3	9.9	10.6	7.6	62.5	93.7	0.66	0.36	1.97	0.49	0.21	0.07
4.7	4.7	4.7	4.2	42.6	33.2	0.77	0.43	1.52	0.35	0.12	0.05
4.5	9.4	8.7	6.6	64.2	87.6	0.68	0.36	1.94	0.42	0.18	0.05
5.0	16.0	7.3	11.7	590.4	273.2	0.57	0.25	2.44	0.65	0.17	0.08
4.9	11.7	8.1	8.0	69.3	95.6	0.63	0.34	1.98	0.42	0.25	0.06
5.0	4.3	5.5	7.7	88.0	71.9	0.63	0.39	1.86	0.54	0.12	0.05
5.0	7.8	7.2	6.9	24.3	31.2	0.65	0.44	1.49	0.55	0.17	0.07
4.8	11.2	6.6	14.7	103.4	129.5	0.54	0.33	2.11	0.5	0.29	0.09
5.3	10.9	8.8	11.3	248.4	156.1	0.56	0.29	2.19	0.62	0.18	0.08
5.3	9.0	8.6	7.9	133.4	130.7	0.62	0.32	2.12	0.14	0.09	0.03
5.5	12.1	12.5	7.3	30.6	46.9	0.62	0.39	1.67	0.73	0.34	0.11
4.3	9.0	7.0	5.4	48.2	37.1	0.73	0.38	1.57	0.37	0.11	0.04
5.1	8.5	7.7	18.0	483.2	297.6	0.51	0.28	2.47	1.1	0.61	0.45
4.9	10.7	7.3	19.7	292.4	149.1	0.50	0.29	2.17	0.51	0.08	0.04

Placebo Group post intervention

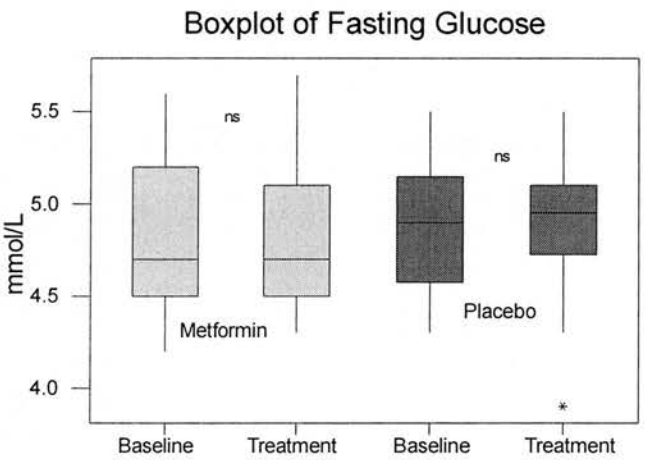
gluc 0 mmol/l	gluc 60 mmol/l	gluc 120 mmol/l	ins 0 mu/l	ins 60 mu/l	ins 120 mu/l	quicki 0 units	quicki 60 units	quicki 120 units	NEFA 0 mmol/l	NEFA 60 mmol/l	NEFA 120 mmol/l
4.9	8.1	6.5	10.4	44.2	55.2	0.59	0.39	0.39	0.43	0.05	0.02
4.4	7.9	8.2	4.0	44.8	120.9	0.80	0.39	0.33	0.71	0.12	0.09
4.8	10.7	8.0	35.1	906.4	705.2	0.45	0.25	0.27	0.49	0.08	0.04
4.9	8.5	7.6	8.7	70.4	136.0	0.61	0.36	0.33	0.52	0.22	0.06
4.9	7.8	7.0	4.0	140.0	80.3	0.77	0.33	0.36	0.48	0.10	0.05
5.2	9.9	11.5	3.1	39.8	43.5	0.83	0.39	0.37	0.59	0.16	0.04
3.9	10.3	6.6	6.9	79.4	33.4	0.70	0.34	0.43	0.72	0.17	0.07
5.5	11.7	9.5	6.5	58.0	88.0	0.64	0.35	0.34	0.74	0.31	0.04
5.1	9.6	7.2	11.0	69.2	32.3	0.57	0.35	0.42	0.37	0.18	0.13
4.8	3.2	4.8	6.5	25.1	75.4	0.67	0.52	0.39	0.4	0.04	0.04
4.8	8.1	6.7	10.7	60.0	56.6	0.58	0.37	0.39	0.42	0.16	0.10
5.0	14.8	4.6	9.2	768.4	72.2	0.60	0.25	0.40	0.78	0.12	0.07
4.3	7.3	5.7	13.4	79.3	34.9	0.57	0.36	0.44	0.14	0.05	0.05
5.1	5.7	6.4	6.5	166.9	41.5	0.66	0.34	0.41	0.44	0.16	0.05
5.0	9.5	6.8	7.8	72.6	65.2	0.63	0.35	0.38	0.37	0.14	0.03
4.5	11.6	10.1	10.2	87.7	179.8	0.60	0.33	0.31	0.37	0.23	0.09
5.3	11.9	10.7	12.6	172.0	181.8	0.55	0.30	0.30	0.66	0.27	0.07
5.1	10.1	8.0	6.4	107.8	169.7	0.66	0.33	0.32	0.46	0.12	0.03
5.4	10.9	6.6	29.7	132.0	73.3	0.45	0.32	0.37	0.46	0.09	0.05
4.4	7.6	10.1	10.2	38.0	56.1	0.61	0.41	0.36	0.23	0.10	0.05
5.0	10.7	9.8	12.0	242.7	403.6	0.56	0.29	0.28	0.87	0.41	0.18
5.1	11.5	6.3	26.8	698.8	186.1	0.47	0.26	0.33	0.38	0.08	0.06

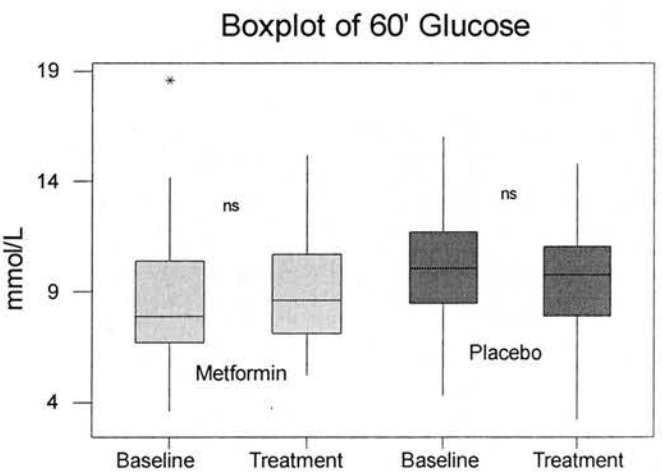
a) Glucose results

Results of the glucose values obtained from the oral glucose tolerance test are summarised in table 7.3 below.

Table 7.3: showing mean change in glucose data before and after intervention
†*Mean +/- standard error of mean*

	Metformin group at baseline†	Metformin group after treatment†	p	Mean change	Placebo group at baseline†	Placebo group after treatment†	p	Mean change	Difference between mean change
Fasting glucose (mmol/L)	4.88 +/- 0.088	4.81 +/- 0.078	ns	-0.067 +/- 0.084	4.87 +/- 0.077	4.88 +/- 0.082	ns	+0.009 +/- 0.068	ns
60 min glucose (mmol/L)	8.97 +/- 0.69	9.29 +/- 0.59	ns	+0.3 +/- 0.443	9.92 +/- 0.56	9.43 +/- 0.52	ns	-0.491 +/- 0.367	ns
120 min glucose (mmol/L)	7.34 +/- 0.48	7.20 +/- 0.52	ns	+0.55 +/- 0.795	7.87 +/- 0.36	7.67 +/- 0.40	ns	-0.2 +/- 0.467	ns



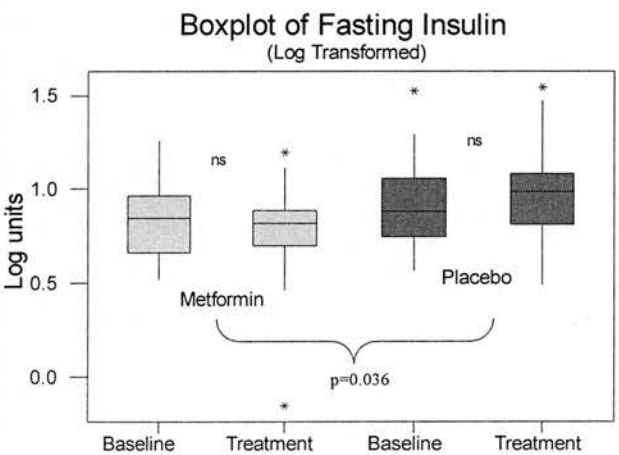


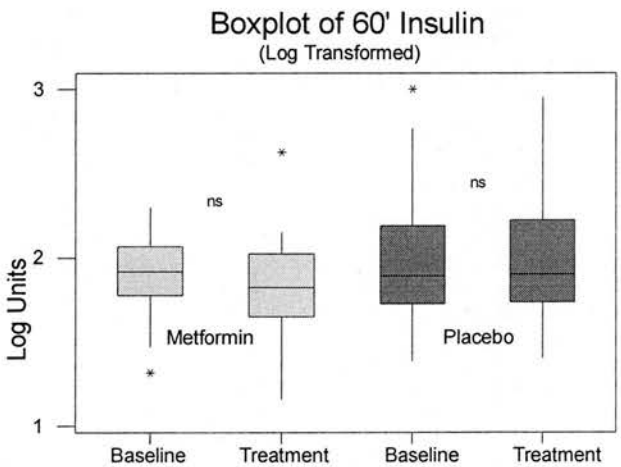
b) Insulin results

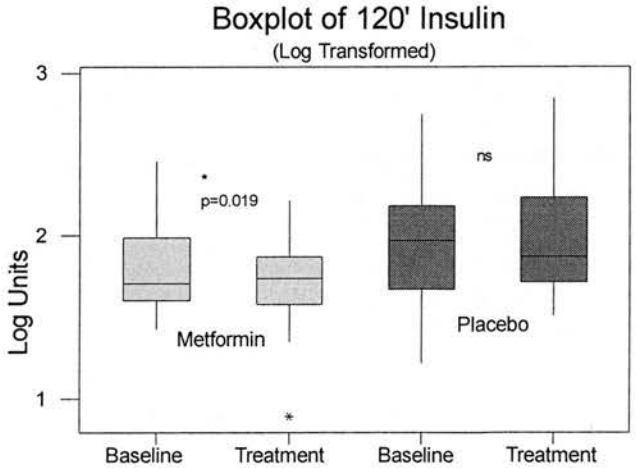
Results of the insulin (log transformed) values obtained from the oral glucose tolerance test are summarised in table 7.4 below.

Table 7.4: showing mean change in insulin data before and after intervention
†Mean +/- standard error of mean

	Metformin group at baseline†	Metformin group after treatment†	p	Mean change	Placebo group at baseline†	Placebo group after treatment†	p	Mean change	Difference between mean change
Fasting log (insulin)	0.845 +/- 0.043	0.771 +/- 0.056	ns	-0.075 +/- 0.047	0.917 +/- 0.050	0.974 +/- 0.057	ns	+0.057 +/- 0.039	**0.036**
60 min log (insulin)	1.894 +/- 0.053	1.822 +/- 0.063	ns	-0.071 +/- 0.050	2.002 +/- 0.089	2.025 +/- 0.091	ns	+0.023 +/- 0.053	ns
120 min log (insulin)	1.800 +/- 0.055	1.703 +/- 0.057	**0.019**	-0.097 +/- 0.038	1.955 +/- 0.078	1.956 +/- 0.075	ns	+0.001 +/- 0.06	ns





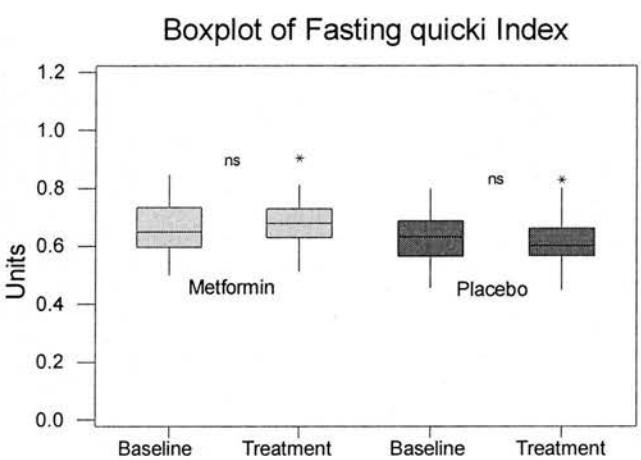


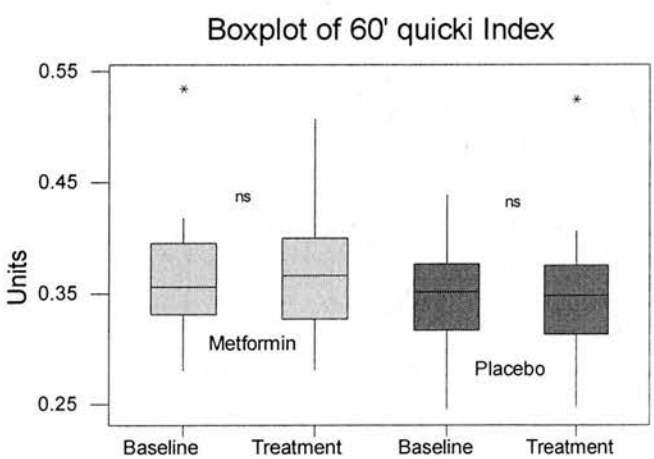
c) Results for Quicki Index of Insulin Resistance

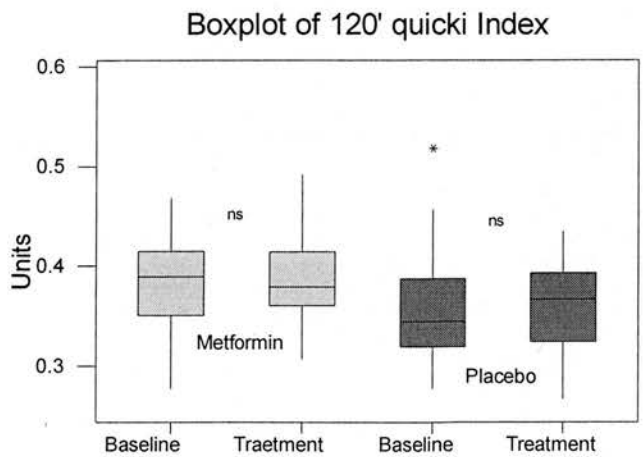
Results of Quicki index obtained from the oral glucose tolerance test are summarised in table 7.5 below.

Table 7.5: showing mean change in quicki index data before and after intervention
†Mean +/- standard error of mean

	Metformin group at baseline†	Metformin group after treatment†	p	Mean change	Placebo group at baseline†	Placebo group after treatment†	p	Mean change	Difference between mean change
Fasting quicki (units)	0.666 +/- .020	0.735 +/- 0.058	ns	+0.068 +/- 0.054	0.636 +/- 0.019	0.617 +/- 0.021	ns	-0.019 +/- 0.014	ns
60 min quicki (units)	0.361 +/- 0.011	0.367 +/- 0.010	ns	+0.006 +/- 0.011	0.343 +/- 0.011	0.345 +/- 0.013	ns	+0.002 +/- 0.009	ns
120 min quicki (units)	0.384 +/- 0.010	0.404 +/- 0.018	ns	+0.035 +/- 0.034	0.360 +/- 0.012	0.360 +/- 0.010	ns	+0.001 +/- 0.011	ns





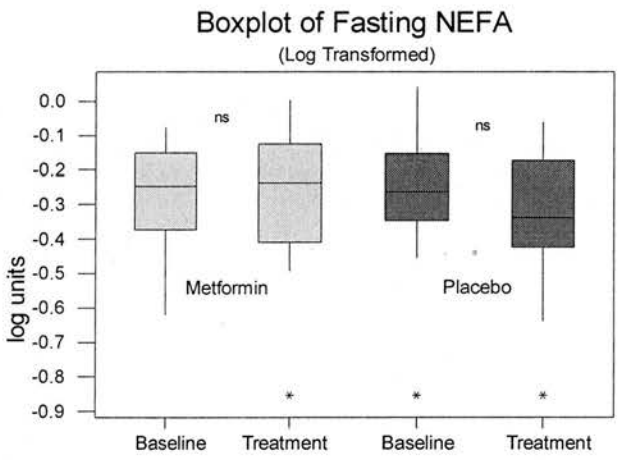


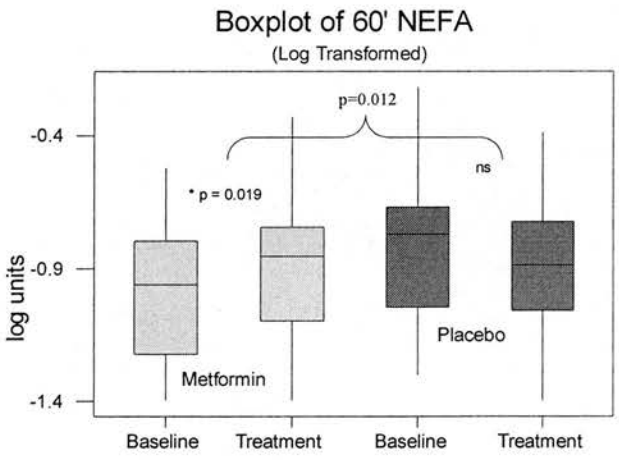
d) Non-esterified fatty acid (NEFA) results

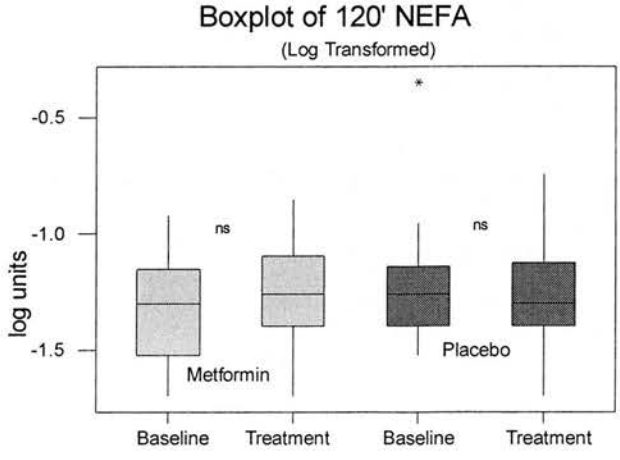
Results of NEFA obtained from the oral glucose tolerance test are summarised in table 7.6 below.

Table 7.6: showing mean change in NEFA data before and after intervention
†*Mean +/- standard error of mean*

	Metformin group at baseline†	Metformin group after treatment†	p	Mean change	Placebo group at baseline†	Placebo group after treatment†	p	Mean change	Difference between mean change
Fasting NEFA (log units)	-0.271 +/- 0.029	-0.0283 +/- 0.039	ns	-0.004 +/- 0.027	-0.267 +/- 0.038	-0.331 +/- 0.038	ns	-0.064 +/- 0.039	ns
60 min NEFA (log units)	-1.006 +/- 0.057	-0.863 +/- 0.053	**0.019**	+0.143 +/- 0.056	-0.824 +/- 0.054	-0.887 +/- 0.055	ns	-0.062 +/- 0.055	**0.012**
120 min NEFA (log units)	-1.344 +/- 0.046	-1.261 +/- 0.046	ns	+0.066 +/- 0.055	-1.220 +/- 0.053	-1.249 +/- 0.047	ns	-0.028 +/- 0.043	ns







Analysis of Glucose/Insulin Metabolism Data

The only index of insulin resistance which showed significant change after metformin treatment compared to placebo was the 2 hour post-glucose load insulin level which was reduced on average just over 5% (p=0.019) after metformin compared to a negligible change with placebo. There was also a significant difference in the change in fasting insulin, between metformin and placebo treated groups. There was an almost 9% decrease in log (fasting insulin) with the metformin group compared to a 6% increase in the placebo group, giving a statistically significant difference (p=0.036).

A similar trend was seen with the 1 hour-post glucose insulin levels with a decrease of almost 4%. This is as compared to placebo in which there was little appreciable change at the 1-hour post-glucose insulin point. This trend at the 1-hour post-glucose insulin level failed to achieve statistical significance.

The glucose levels were relatively unaffected after metformin treatment with an approximate reduction in the fasting glucose of 1.5%. Although this trend was in a favourable direction, statistical significance was not reached.

Looking at the quicki index of insulin sensitivity, a 10% increase at the fasting state was seen after metformin compared to a slight reduction in those taking placebo. However, this missed out on statistical significance (p=0.215). A 9% increase at the 2-our post glucose point was also seen compared to a negligible change for placebo but again this insufficient to obtain significance (p=0.115).

These patterns point to very modest improvements in insulin resistance in this group after metformin treatment with most parameters moving in a favourable direction but only 2-hour post glucose load insulin reaching significant levels of amelioration.

2 : Effects of Metformin on Lipid Profile

The raw data are shown in the tables below for the metformin and placebo groups at baseline and then post intervention.

Metformin group at baseline

total chol mmol/l	trig mmol/l	LDL-C mmol/l	HDL-C mmol/L
6.50	1.85	4.60	1.20
5.45	1.30	2.85	2.25
4.45	1.05	2.85	1.30
5.60	1.10	3.30	1.60
5.60	1.90	3.50	1.75
5.75	1.95	4.00	1.05
5.80	1.40	3.65	1.20
4.30	0.85	2.50	1.55
5.40	2.20	3.25	1.30
4.30	1.00	2.55	1.55
6.55	0.75	4.15	1.85
6.30	1.40	3.60	2.05
4.30	2.15	2.70	0.80
4.55	0.85	2.75	1.50
5.80	1.60	3.80	1.80
5.10	1.60	3.40	1.05
3.50	1.70	1.70	1.40
4.65	1.25	2.95	1.35
3.90	1.15	2.55	0.85
3.65	1.15	2.15	1.10
3.90	0.80	2.35	1.25
4.20	1.00	2.55	1.30
4.10	1.00	2.65	1.15
4.30	2.70	2.25	1.35

Metformin group post intervention

total chol mmol/l	trig mmol/l	LDL-C mmol/l	HDL-C mmol/L
5.70	1.60	4.05	1.25
4.75	1.55	2.40	2.20
4.15	0.85	2.30	1.50
4.75	0.70	2.80	1.80
6.05	2.20	3.35	1.90
6.85	1.95	4.85	1.25
5.50	0.90	3.45	1.90
4.80	0.85	2.75	1.90
4.90	1.20	3.35	1.45
4.85	0.75	2.95	1.55
6.50	0.90	4.45	2.00
5.40	1.00	2.80	2.25
3.55	1.35	2.20	0.95
3.50	0.90	1.95	1.30
5.50	1.35	3.20	1.90
5.30	1.85	3.55	1.10
4.95	2.10	2.90	1.35
5.70	1.25	3.65	1.35
4.30	1.25	3.00	0.90
4.90	1.70	3.00	1.35
3.40	0.80	1.98	1.05
3.85	0.85	2.15	1.20
4.30	1.25	2.60	1.25
4.35	2.00	2.45	1.50

Placebo group at baseline

total chol mmol/l	trig mmol/l	LDL-C mmol/l	HDL-C mmol/L
5.45	1.20	3.75	1.45
6.95	1.60	5.10	1.45
3.45	1.25	2.10	1.05
5.75	1.65	4.15	1.15
4.80	0.90	2.90	1.50
4.75	1.30	3.30	1.40
5.25	2.90	3.00	1.10
4.75	1.45	3.20	1.30
4.20	1.20	2.80	1.20
5.90	0.70	4.15	1.45
4.15	1.10	2.60	1.20
3.45	1.40	1.35	1.80
5.65	0.85	3.95	1.20
4.45	0.95	2.70	1.50
4.70	1.05	3.00	1.35
4.60	1.70	3.00	1.20
5.20	3.30	3.15	0.95
3.60	0.90	2.30	1.15
5.25	1.55	2.45	2.40
4.55	1.10	2.95	1.10
7.60	17.70	1.30	0.75
3.70	1.40	2.25	1.10

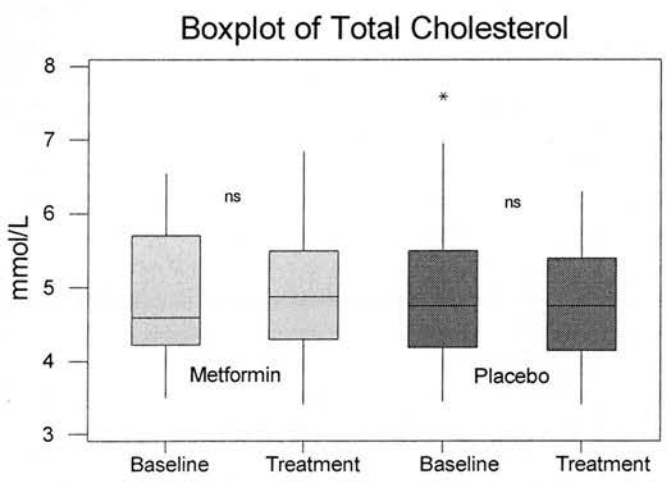
Placebo group post intervention

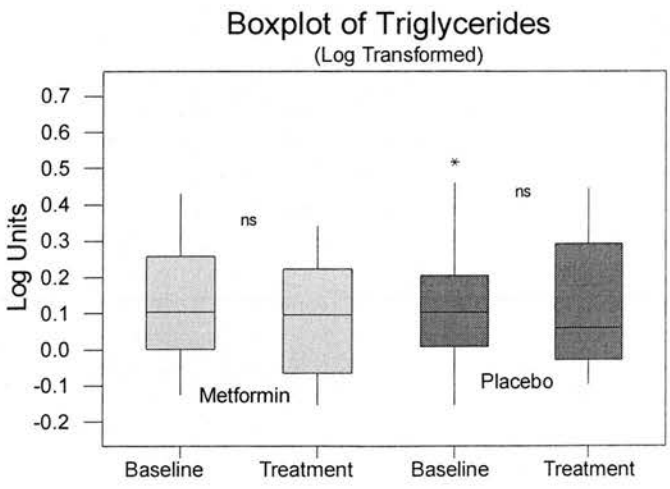
total chol mmol/l	trig mmol/l	LDL-C mmol/l	HDL-C mmol/L
4.55	0.90	3.05	1.35
6.30	1.15	4.60	1.40
3.40	1.05	2.00	1.05
4.10	1.25	2.55	1.00
4.70	0.80	3.10	1.50
4.80	1.15	3.40	1.20
5.70	2.70	3.60	1.20
4.55	1.95	2.70	1.20
5.40	2.00	3.25	1.25
5.40	0.85	3.90	1.30
4.40	0.95	2.75	1.20
4.00	1.60	1.65	2.05
5.40	1.00	3.75	1.15
4.70	0.90	2.95	1.55
4.90	1.25	3.10	1.55
4.95	2.45	2.95	1.20
5.40	2.80	3.15	1.05
3.50	1.00	2.05	1.15
5.20	0.90	3.15	2.20
3.70	1.15	2.35	1.10
6.20	10.20	1.75	1.00
4.15	1.30	2.55	1.35

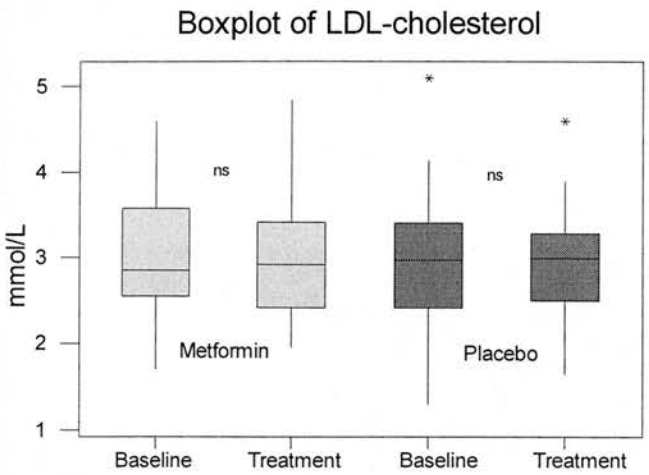
Beta-quant analysis was undertaken in the fasting state pre and post treatment with placebo and metformin. Before and after data are shown for these with respect to placebo and metformin in table 7.7 below and in the boxplots following.
As described earlier, triglyceride values required log transformation to achieve normality but the other lipid variables did not.

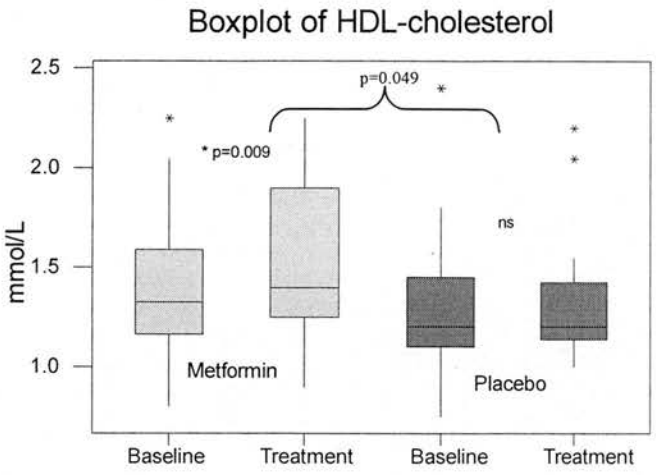
Table 7.7: showing mean change in lipid data before and after intervention
†Mean +/- standard error of mean

	Metformin group at baseline†	Metformin group after treatment†	p	Mean change	Placebo group at baseline†	Placebo group after treatment†	p	Mean change	Difference between mean change
Total cholesterol (mmol/L)	4.91 +/- 0.19	4.91 +/- 0.18	ns	-0.006 +/- 0.15	4.92 +/- 0.22	4.79 +/- 0.17	ns	-0.125 +/- 0.14	ns
Triglycerides (log mmol/L)	0.121 +/- 0.03	0.086 +/- 0.03	ns	-0.036 +/- 0.02	0.166 +/- 0.062	0.148 +/- 0.054	ns	-0.02 +/- 0.025	ns
LDL-cholesterol (mmol/L)	3.03 +/- 0.14	3.01 +/- 0.15	ns	-0.020 +/- 0.11	2.98 +/- 0.19	2.92 +/- 0.15	ns	-0.05 +/- 0.11	ns
HDL-cholesterol (mmol/L)	1.40 +/- 0.07	1.51 +/- 0.08	**0.009**	+0.11 +/- 0.04	1.31 +/- 0.07	1.32 +/- 0.07	ns	+0.01 +/- 0.03	**0.049**









Analysis of Lipid Data

No appreciable difference was seen in either total or LDL-cholesterol after metformin treatment. However, HDL-cholesterol did increase significantly on average by almost 8% (p=0.009) compared to an average increase of less than 1% in those taking placebo. The difference in change between the 2 groups also just achieved statistical significance for HDL-cholesterol (p=0.049). A favourable trend also emerged from log-transformed triglyceride analysis with an average reduction of just over 29% in those receiving metformin compared to a reduction of approximately 11% in those taking placebo. These reductions were not statistically significant.

From the women treated with metformin, 13 out of 24 (54%) were on a statin prior to and during the trial. This compares with 7 out of 22 (32%) for the placebo-treated women. These differences as judged by 2x2 chi-squared test are not significant (p=0.127).

From these data significant increases in HDL-cholesterol were seen in the metformin-treated group with a favourable trend emerging from the triglyceride data. No appreciable change is seen in total or LDL-cholesterol. This is despite no significant difference in statin usage between the groups.

3 : Effects of Metformin on Serum Endothelial Markers

The raw data are shown in the tables below for the metformin and placebo groups at baseline and then post intervention.

Metformin group at baseline

vWF %	tPA ng/L	ICAM ng/ml	VCAM ng/l	D-dimer units
247	9.2	283.4	308.7	67
138	4.3	250.9	234.7	85
210	5.9	284.3	383.7	67
103	5.3	300.2	456.9	47
131	2.0	185.8	247.2	47
95	6.7	233.6	352.2	84
171	12.1	284.7	396.4	422
163	6.5	283.4	587.6	103
124	10.5	439.8	302.5	38
123	3.8	220.1	444.6	83
121	4.9	201.3	279.2	56
128	6.5			126
168	13.1	362.0	380.6	62
196	10.5	262.2	392.9	109
57	4.3	206.0	300.1	101
141	9.9	405.7	348.2	84
73	7.4	266.1	279.2	31
66	6.3	202.1	327.5	59
178	10.0	196.5	323.1	56
95	9.6	196.8	239.5	165
137	3.8	309.9	488.9	32
67	6.4	230.6	395.6	110
100	11.2	190.4	269.6	353
66	7.5	276.7	433.9	84

Metformin group post intervention

vWF %	tPA ng/L	ICAM ng/ml	VCAM ng/l	D-dimer units
240	6.6	237.9	400.5	80
132	2.8	209.4	271.8	63
194	4.6	263.2	341.4	86
110	5.8	308.9	412.9	43
134	2.7	187.9	273.2	37
89	5.2	231.7	332.3	75
194	9.5	245.6	408.6	335
144	4.8	211.6	320.3	1019
94	6.0	491.3	347.2	45
116	3.9	197.0	429.4	108
110	3.8	205.9	270.2	46
148	7.1	315.6	415.2	82
167	7.1	263.9	265.2	100
51	2.2	204.5	211.5	63
118	8.2	416.9	423.5	99
73	7.4	257.2	310.0	28
74	5.6	178.4	398.3	34
173	9.8	219.9	264.6	60
98	5.8	206.6	207.0	183
123	4.1	257.8	432.5	41
83	6.1	213.4	392.3	102
111	11.1	284.8	546.3	302
65	7.7	251.1	420.5	90

Placebo group at baseline

vWF %	tPA ng/L	ICAM ng/ml	VCAM ng/l	D-dimer units
130	9.6	229.2	298.2	61
131	6.7	180.4	230.4	38
128	11.3	289.8	394.9	64
165	11.6	222.3	259.8	47
156	6.7	216.1	272.2	24
117	6.9	241.3	238.4	112
170	7.7	293.3	288.8	68
148	4.0	261.5	270.6	84
91	14.7	284.0	352.1	174
136	6.2	161.6	305.7	48
88	7.0	228.2	352.2	106
158	7.0	176.4	374.9	34
107	10.0	213.1	293.7	45
141	6.5	179.1	347.2	222
96	7.9	221.5	390.3	117
101	9.9	202.9	240.9	194
140	12.8	259.3	258.6	86
109	6.7	212.4	374.4	64
171	12.1	228.1	325.3	163
184	7.4	438.4	589.0	268
55	8.0	294.7	408.8	52
301	10.4	265.2	335.4	51

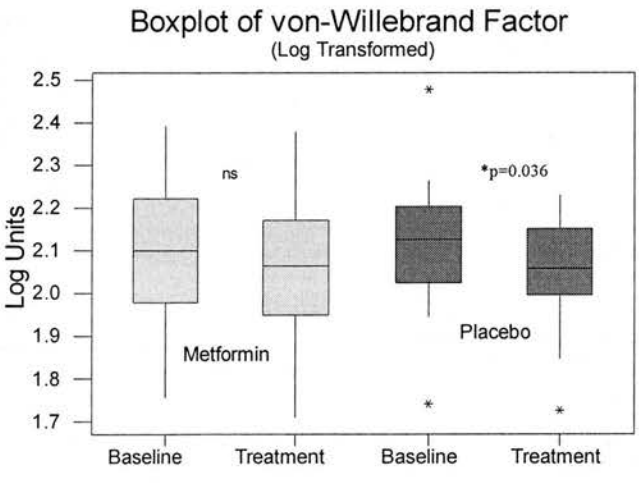
Placebo group post intervention

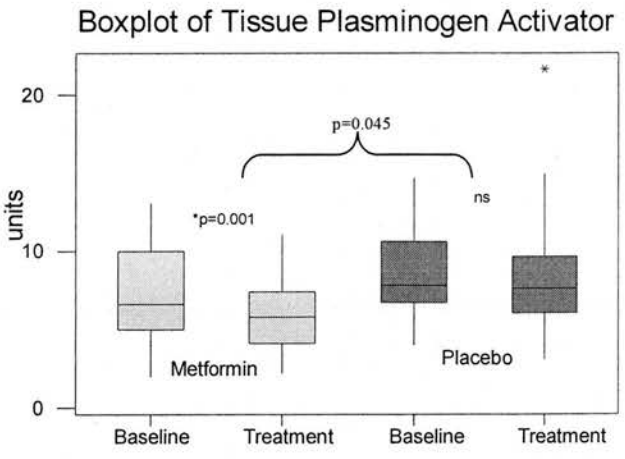
vWF %	tPA ng/L	ICAM ng/ml	VCAM ng/l	D-dimer units
120	8.7	211.1	291.1	57
110	6.0	186.7	280.5	30
98	10.1	263.1	380.5	57
141	7.5	208.5	359.2	52
147	6.1	214.3	303.3	27
92	8.5	231.3	261.5	126
205	8.7	251.1	315.9	59
158	5.7	246.8	285.2	108
110	21.7	277.8	349.1	121
136	3.1	176.7	248.9	61
85	8.9	264.2	353.6	60
143	7.7	185.4	359.8	48
111	5.6	287.8	280.4	78
148	6.6	172.8	315.6	167
104	4.2	213.8	365.2	45
70	6.5	218.6	249.6	130
117	13.8	262.6	289.5	71
108	7.4	195.0	318.5	54
121	15.0	243.3	351.2	86
140	7.6	404.7	471.5	62
53	9.5	281.1	363.6	45
99	11.0	302.9	315.0	55

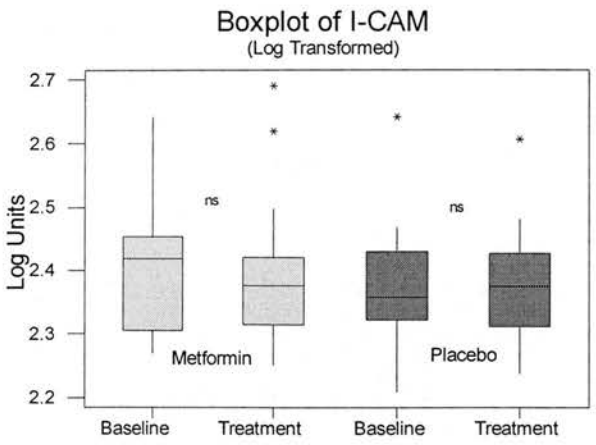
Venous samples were obtained in the fasting state pre and post treatment with placebo and metformin. Before and after data are shown in table 7.8 for these with respect to placebo and metformin with boxplots thereafter. As described in the methods section, all endothelial markers except tPA required log transformation to become normally distributed.

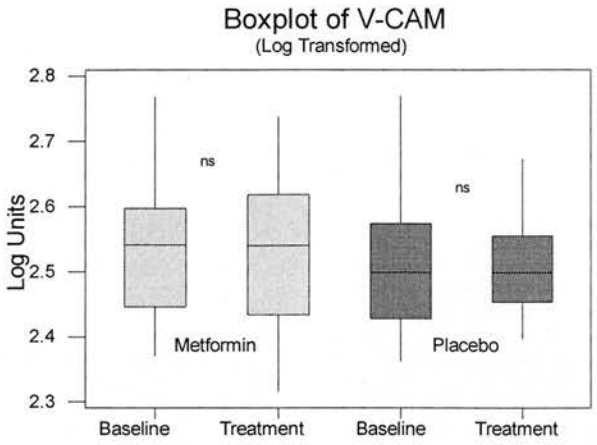
Table 7.8: showing mean change in serum endothelial cell marker data before and after intervention
†*Mean +/- standard error of mean*

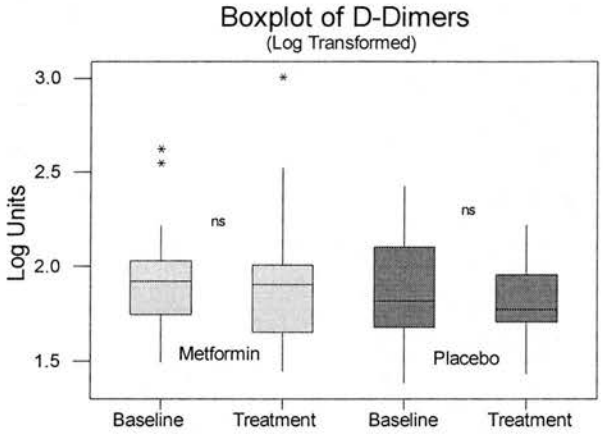
	Metformin group at baseline†	Metformin group after treatment†	p	Mean change	Placebo group at baseline†	Placebo group after treatment†	p	Mean change	Difference between mean change
vWF (log units)	2.078 +/- 0.037	2.062 +/- 0.034	ns	-0.016 +/- 0.01	2.115 +/- 0.031	2.059 +/- 0.027	**0.036**	-0.056 +/- 0.025	ns
tPA (units)	7.404 +/- 0.60	5.996 +/- 0.48	**0.001**	-1.448 +/- 0.36	8.686 +/- 0.56	8.632 +/- 0.86	ns	-0.055 +/- 0.562	**0.045**
I-CAM (log units)	2.409 +/- 0.022	2.392 +/- 0.022	ns	-0.017 +/- 0.01	2.371 +/- 0.020	2.373 +/- 0.019	ns	+ 0.002 +/- 0.01	ns
V-CAM (log units)	2.538 +/- 0.022	2.534 +/- 0.023	ns	-0.005 +/- 0.02	2.504 +/- 0.021	2.504 +/- 0.014	ns	+0.000 +/- 0.01	ns
D-dimer (log units)	1.910 +/- 0.057	1.930 +/- 0.075	ns	+0.028 +/- 0.05	1.894 +/- 0.060	1.816 +/- 0.043	ns	-0.078 +/- 0.042	ns











Analysis of Serum Endothelial Marker Data

Only tPA showed a significant change after treatment with metformin – a reduction of 19% compared to little appreciable change in the placebo-treated group. Although tPA also showed a non-significant decrease in the placebo group, the difference between the change in the 2 groups for tPA also achieved statistical significance (p=0.045). Weak trends towards reduction were seen with log-transformed vWF and I-CAM although for some of these parameters similar trends towards a decrease were observed in the placebo-treated group.

Of note, there was a significant decrease in vWF of approximately 3% after placebo (p=0.036). The reason for this is unclear but likely reflects statistical variability due to the relatively small group sizes.

Only tPA showed a significant downward difference after treatment with metformin.

4 : Effects of Metformin on Blood Pressure

The raw data for the averaged blood pressures are shown in the tables below for the metformin and placebo groups at baseline and then post intervention.

Metformin group

Systolic BP Baseline mmHg	Systolic BP Post mmHg		Diastolic BP Baseline mmHg	Diastolic BP Post mmHg
126	139		75	77
111	116		73	69
128	118		79	71
156	145		85	79
151	134		87	69
121	138		71	78
176	183		103	106
136	114		82	74
143	134		76	78
127	123		85	85
133	143		74	97
137	141		92	87
110	95		67	55
148	141		77	80
153	155		83	87
129	117		82	64
92	101		70	77
152	149		85	82
121	127		72	70
142	126		90	90
112	107		71	83
103	95		75	59
111	117		73	72
133	118		82	74

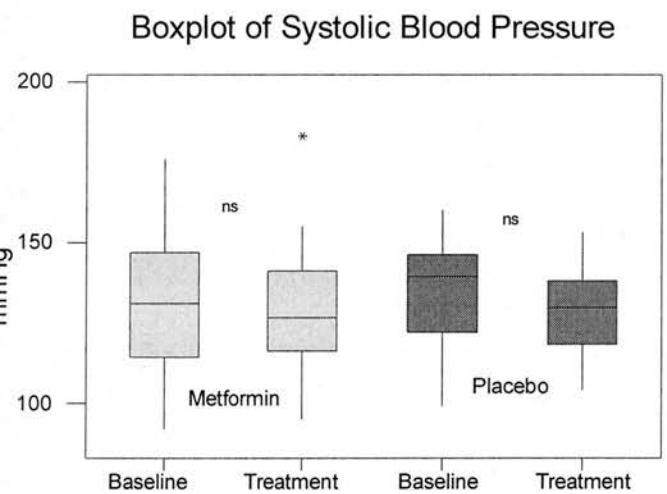
Placebo group

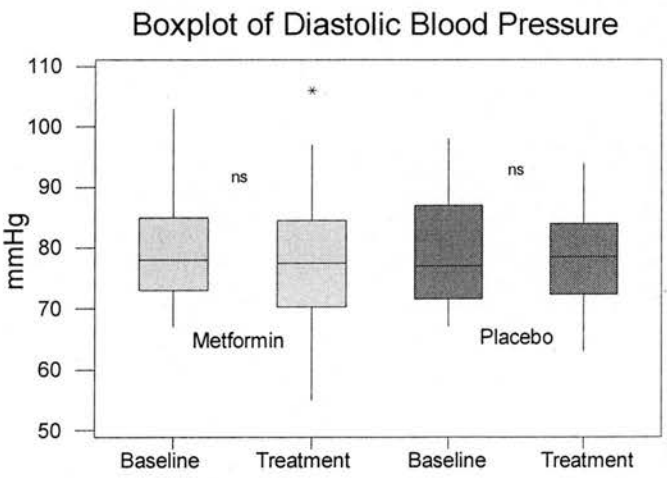
Systolic BP Baseline mmHg	Systolic BP Post mmHg		Diastolic BP Baseline mmHg	Diastolic BP Post mmHg
135	126		86	82
99	121		67	70
139	105		67	63
135	125		97	80
134	119		75	64
157	253		93	86
146	138		78	75
119	114		73	73
148	119		87	78
149	151		85	82
140	135		87	79
160	153		85	83
108	104		69	65
123	116		76	69
125	138		70	74
144	137		87	84
141	137		72	78
119	133		75	88
144	135		98	91
141	126		73	84
146	151		91	94
115	107		70	73

Averaged systolic and diastolic measurements made before and after administration of metformin or placebo. Before and after data are shown in table 7.9 with boxplots thereafter.

Table 7.9: showing mean change in blood pressure before and after intervention
†Mean +/- standard error of mean

	Metformin group at baseline†	Metformin group after treatment†	p	Mean change	Placebo group at baseline†	Placebo group after treatment†	p	Mean change	Difference between mean change
Systolic BP (mmHg)	131.3 +/- 4.0	128.2 +/- 4.1	ns	-3.1 +/- 2.2	134.9 +/- 3.3	133.8 +/- 6.4	ns	-1.1 +/- 5.3	ns
Diastolic BP (mmHg)	79.5 +/- 1.7	77.6 +/- 2.3	ns	-1.9 +/- 1.9	80.0 +/- 2.1	78.0 +/- 1.8	ns	-2.1 +/- 1.5	ns





Analysis of Blood Pressure Data

Small downward trends are observed for both systolic and diastolic blood pressure in those treated with metformin and placebo. No difference achieved statistical significance, nor did the difference between the changes in respective groups. In summary, no significant effect on blood pressure was observed with metformin treatment.

5 : Effects of Metformin on C-Reactive Protein

The raw data are shown in the tables below for the metformin and placebo groups at baseline and then post intervention.

Metformin group

CRP mg/l baseline	CRP mg/l post
2.95	1.72
5.68	5.06
2.53	3.54
2.02	1.06
7.83	6.42
1.47	0.63
0.92	0.86
5.82	5.35
2.80	1.29
3.19	7.66
1.54	2.03
4.06	
5.62	4.65
2.75	2.37
2.83	1.98
1.64	2.05
4.44	8.49
1.90	0.93
0.56	0.63
4.12	2.98
4.81	23.68
2.39	3.43
1.09	0.76
9.03	11.84

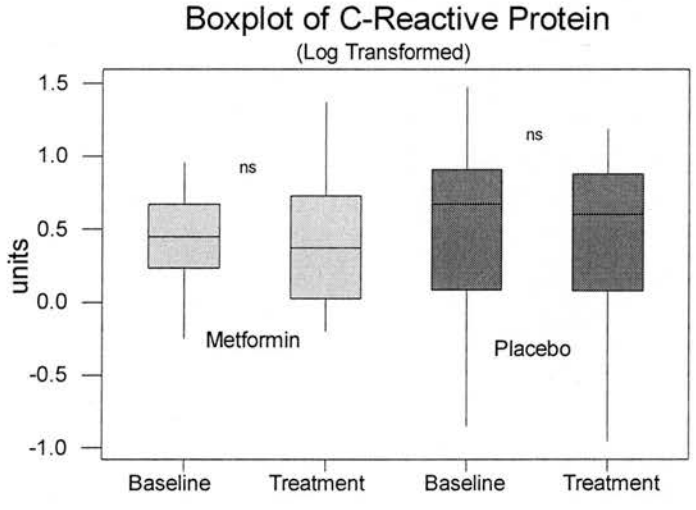
Placebo group

CRP mg/l baseline	CRP mg/l post
0.33	0.31
0.50	0.35
13.46	15.56
2.74	1.19
1.18	1.20
10.04	8.89
10.17	10.60
5.41	4.01
4.77	3.66
0.14	0.11
2.34	3.32
4.61	7.18
6.84	6.18
0.26	0.41
1.23	2.43
4.25	5.26
4.38	3.96
5.63	5.95
8.13	10.22
30.08	3.96
7.98	7.52
8.12	7.59

Venous samples were obtained in the fasting state before and after treatment with metformin/placebo and analysed for high sensitivity C-reactive protein, as a measure of levels of inflammation. Before and after data are shown in table 7.10 with boxplots thereafter. CRP data required log transformation in order to be distributed normally.

Table 7.10: showing mean change in C-reactive protein before and after intervention
†*Mean +/- standard error of mean*

	Metformin group at baseline†	Metformin group after treatment†	p	Mean change	Placebo group at baseline†	Placebo group after treatment†	p	Mean change	Difference between mean change
C-reactive Protein (log mg/L)	0.445 +/- 0.061	0.425 +/- 0.090	ns	-0.013 +/- 0.05	0.501 +/- 0.13	0.463 +/- 0.12	ns	-0.038 +/- 0.05	ns



Analysis of CRP data

There appears to be no significant effect of metformin on CRP with a small average fall (3%) in the log-transformed values compared to an almost 8% reduction after placebo. There is no significant difference between the changes seen in both groups. **In summary metformin appears to have no significant effect on CRP after 8 weeks of treatment.**

6 : Effect of Metformin on Anthropometric Variables

The raw data are shown in the tables below for the metformin and placebo groups at baseline and then post intervention.

Metformin group at baseline

Waist (cm)	W:H ratio	Weight (kg)	BMI (Kg/m^2)	Leptin (ng/ml)
85.0	0.83	62.0	25.00	37.9
82.0	0.75	69.0	25.70	28.8
69.0	0.7	59.0	23.60	19.3
92.0	0.79	81.0	29.60	35.4
90.0	0.83	70.0	32.20	40.8
78.0	0.76	65.0	24.50	23.4
79.0	0.77	69.0	28.40	23.7
91.0	0.76	80.0	34.20	44.8
77.0	0.78	58.0	24.50	24.8
82.0	0.79	66.0	27.10	44.9
77.0	0.77	65.0	25.20	31.9
102.0	0.85	88.0	37.10	36.4
89.0	0.82	68.0	25.80	16.9
85.0	0.83	65.0	27.90	57.7
87.0	0.85	72.0	27.20	29.7
99.0	0.85	83.0	31.80	41.6
85.0	0.79	71.0	25.40	29.4
76.0	0.76	61.0	23.60	11.2
90.0	0.77	75.0	27.00	38.2
83.0	0.78	73.0	29.40	28.5
84.0	0.75	79.0	32.00	50.9
87.0	0.76	88.0	34.40	52.5
83.0	0.88	62.0	23.00	48.6
100.0	0.76	92.0	34.70	60.6

Metformin group post intervention

Waist (cm)	W:H ratio	Weight (kg)	BMI (Kg/m^2)	Leptin (ng/ml)
82.0	0.83	65.0	27.06	25.5
79.0	0.72	70.0	25.71	25.6
69.0	0.8	57.0	22.55	14.8
93.0	0.81	79.0	28.33	35.4
84.0	0.8	70.0	31.11	33.4
81.0	0.78	66.0	24.84	25.5
83.0	0.78	67.5	27.38	11.8
92.0	0.75	81.5	33.06	59.8
83.0	0.85	59.0	23.94	26.8
84.0	0.8	67.0	27.53	55.2
80.0	0.79	65.5	25.27	40.1
109.0	0.89	87.0	37.41	
85.0	0.79	67.0	24.91	20.4
80.5	0.82	63.0	27.27	26.5
84.0	0.84	69.0	26.29	18.6
91.0	0.8	80.5	31.06	34.4
85.0	0.79	72.5	26.63	28.4
74.0	0.76	62.5	24.57	9.9
86.0	0.79	72.5	26.63	29.2
81.0	0.76	71.5	28.82	33
89.5	0.84	80.0	31.25	53.7
86.0	0.74	85.5	33.19	57.5
82.0	0.87	60.5	22.63	39.4
97.0	0.74	90.5	34.70	61.8

Placebo group at baseline

Waist (cm)	W:H ratio	Weight (kg)	BMI (Kg/m^2)	Leptin (ng/ml)
100.0	0.85	74.0	29.80	58.4
78.0	0.73	66.0	26.40	28.7
112.0	0.99	82.0	37.20	60.2
96.0	0.79	85.0	33.20	49.9
70.0	0.73	55.0	21.60	24.2
81.0	0.72	76.0	30.40	38.4
103.0	0.87	87.0	34.90	38.0
82.0	0.78	70.0	25.50	37.0
89.0	0.81	78.0	28.80	44.3
83.0	0.84	64.0	23.40	15.3
80.0	0.78	66.0	27.60	30.7
79.0	0.78	58.0	25.10	41.3
89.0	0.84	80.0	28.70	47.7
91.0	0.81	77.0	26.60	36.9
91.0	0.79	73.0	28.50	21.3
89.0	0.80	79.0	27.70	21.9
87.0	0.80	68.0	26.20	51.0
79.0	0.79	65.0	26.70	46.4
122.0	0.86	102.0	43.70	49.8
86.0	0.84	68.0	27.70	26.8
92.0	0.82	74.0	27.90	32.6
102.0	0.86	82.0	35.30	52.2

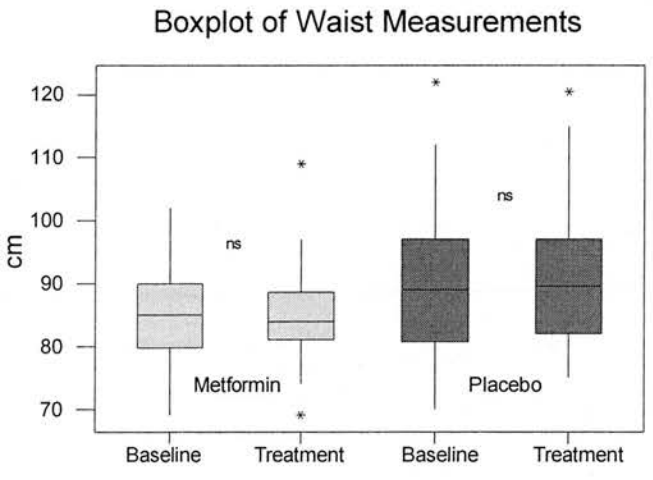
Placebo group post intervention

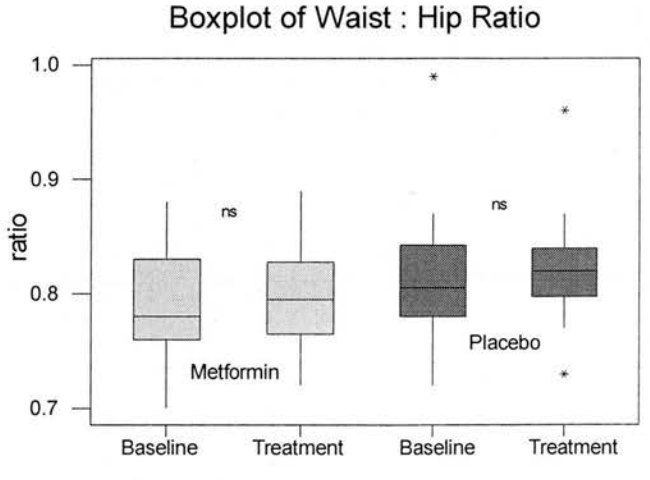
Waist (cm)	W:H ratio	Weight (kg)	BMI (Kg/m^2)	Leptin (ng/ml)
97.0	0.83	77.0	31.64	62.7
89.0	0.83	67.5	27.74	31.6
115.0	0.96	85.0	39.34	69.6
100.0	0.83	83.0	33.67	33.3
75.0	0.77	56.0	22.15	25.9
81.0	0.73	76.5	29.51	25.6
97.0	0.82	88.0	34.81	46.5
79.0	0.77	69.0	25.34	32.4
91.0	0.84	77.0	29.34	51.3
83.0	0.84	65.5	24.06	17.3
82.0	0.80	65.5	27.26	38.1
82.0	0.80	59.5	25.42	39.8
92.0	0.86	79.0	29.02	42.1
87.0	0.81	78.5	27.16	32.6
90.0	0.79	75.0	28.93	32.6
87.0	0.80	80.0	28.18	16.5
85.0	0.79	67.0	25.85	46.6
80.0	0.81	65.5	26.57	43.7
120.5	0.84	104.0	43.85	57.9
94.0	0.87	70.0	29.14	38.9
92.0	0.82	75.0	27.89	34.6
101.0	0.86	82.5	35.71	46.4

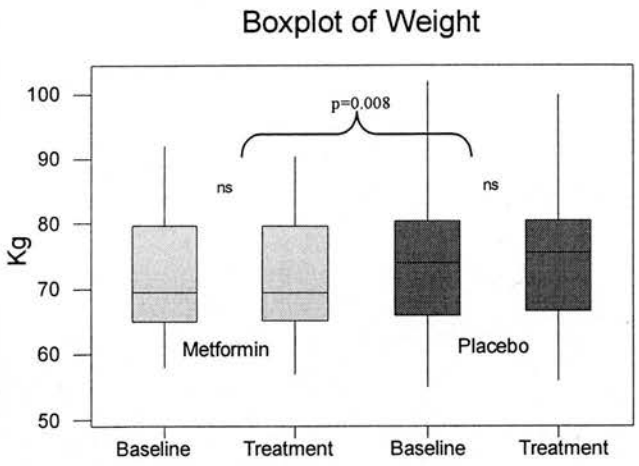
Venous samples were obtained in the fasting state pre and post treatment with placebo and metformin and analysed for leptin. Anthropometric measurements were also made pre and post metformin/placebo. Before and after data are shown in table 7.11 for these with respect to placebo and metformin with boxplots thereafter.

Table 7.11: showing mean change in anthropometric data and serum leptin before and after intervention
†*Mean +/- standard error of mean*

	Metformin group at baseline†	Metformin group after treatment†	p	Mean change	Placebo group at baseline†	Placebo group after treatment†	p	Mean change	Difference between mean change
Waist (cm)	85.5 +/- 1.62	85.0 +/- 1.60	ns	-0.5 +/- 0.79	90.05 +/- 2.58	90.89 +/- 2.39	ns	+0.84 +/- 0.84	ns
Waist:Hip Ratio (units)	0.79 +/- 0.009	0.80 +/- 0.009	ns	+0.007+/-0.008	0.81 +/- 0.012	0.82 +/- 0.010	ns	+0.009 +/- 0.006	ns
Weight (Kg)	71.71 +/- 1.97	71.23 +/- 1.87	ns	-0.48 +/- 0.35	74.05 +/- 2.23	74.82 +/- 2.25	**0.015**	+0.77 +/- 0.29	**0.008**
Body Mass Index (Kg/m^2)	28.30 +/- 0.83	28.01 +/- 0.79	ns	-0.30 +/- 0.17	29.22 +/- 1.06	29.66 +/- 1.09	**0.01**	+0.44 +/- 0.15	**0.003**
Leptin (ng/ml)	35.75 +/- 2.64	33.33 +/- 3.18	ns	-2.38 +/- 2.01	38.77 +/- 2.67	39.36 +/- 2.86	ns	+0.59 +/- 1.64	ns



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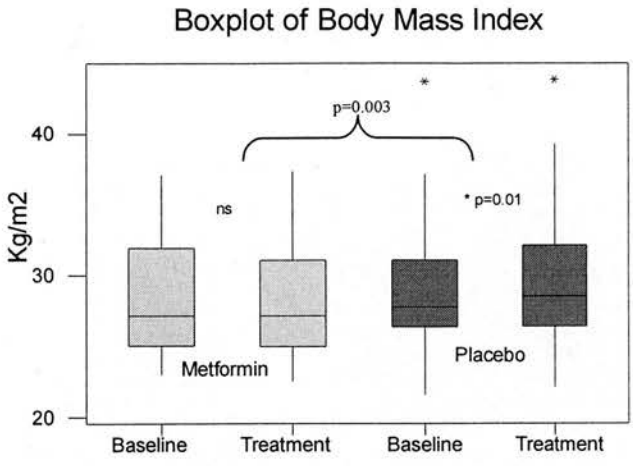
Metformin
Placebo
Baseline
Treatment
Baseline
Treatment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Metformin
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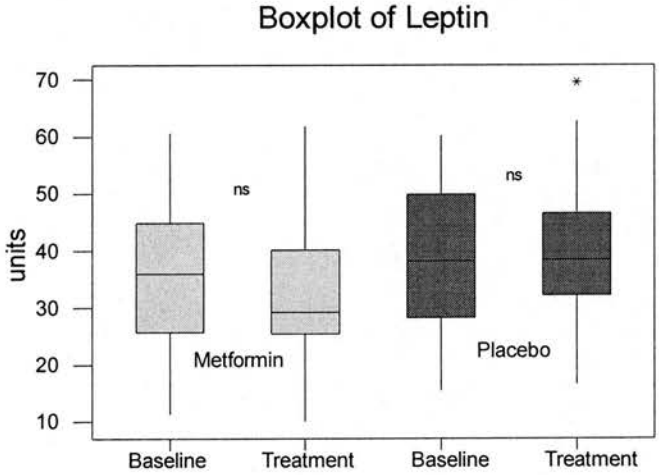
Baseline
Treatment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Metformin
Placebo
Baseline
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Baseline
Treatment

Analysis of Anthropometric Data and Leptin

There is no statistically significant change in any of the anthropometric data after treatment with metformin. However, several non-significant trends have emerged. Waist circumference was reduced by an average of 0.5cm approximately in both the placebo and metformin-treated groups and W:H ratio fell slightly in both groups also.

Treatment with metformin reduced body mass by an average of approximately 0.5kg over 8 weeks compared to an average increase of approximately 0.8kg in the placebo-treated group. This increase in weight in the placebo group was actually statistically significant and was the driving factor behind the difference in mean change in weight being statistically significant between the groups ($p=0.008$). This change in body mass affected the BMI with a small non-significant fall for the metformin group compared to a statistically significant increase in the placebo-treated group ($p=0.01$). Again this increase in the placebo group drove the difference in change between the groups to statistical significance ($p=0.003$).

The reasons behind the significant weight gain and increase in BMI in the placebo-group are unclear.

Leptin was reduced by almost 7% in the metformin group (non-significant) compared to a increase of 1.5% in the placebo group. These trends in leptin are not surprising as serum leptin is known to be correlated to body fat mass.

These figures suggest favourable trends with metformin treatment. Modest reductions occurred in total body mass and leptin (related to fat mass) although it is likely that our sample sizes were insufficiently powered to prove these trends statistically significant.

In summary, metformin treatment was not associated with any significant changes in weight, waist, BMI or leptin but favourable trends towards a reduction in total body and fat mass were seen.

Summary

Metformin is an insulin sensitiser that had a number of actions primarily on the liver. It is fairly well tolerated and of the 27 women randomised to metformin, 24 completed the 8 week trial, compared to 22 of the 29 women randomised to placebo. The main side-effect was mild gastro-intestinal upset. There was a small increase in lactate in both groups and the magnitude of this rise was similar between the metformin and placebo groups. The reason for the increase in lactate in the placebo group is not clear.

The 22 women in the placebo group were well matched to the 24 women in the metformin group in terms of baseline characteristics and baseline measured variables. The only statistically significant difference was that more women in the metformin group were taking calcium-channel blockers during the trial compared to the placebo group (4 out of 24 compared to 0 out of 22). The effect of this on the results is unclear but there is no data to show that calcium channel antagonism affects any of the variables measured.

The insulin/glucose data did point to some modest increases in insulin sensitivity. Although there were not any differences in glucose tolerance, there were important changes in the insulin levels. There were downward trends in measured insulin levels at all 3 points in the oral glucose tolerance test (OGTT), suggesting improved insulin sensitivity. The reduction in insulin became significant at the 120min point of the OGTT but there was a significant difference in the change between the metformin and placebo groups at the fasting sample. No trends emerged within the quicki index, which likely reflects that glucose is taken into account when calculating this index.

The lipid data did show a highly significant increase in HDL-cholesterol in the metformin group with no discernable trend seen in the other lipid variables measured. Looking at the serum endothelial markers, there was a trend towards lower vWF after metformin (as in the placebo group also) but there was a highly significant reduction in tPA. No trends of any importance were seen with I-CAM, V-CAM or d-dimer.

There was a small trend towards lower blood pressure after metformin, which was most evident in the systolic pressure but no statistical significance was reached. Similarly, there was a minor trend towards lower C-reactive protein with metformin (and also in the placebo group) but statistical significance was not achieved. In terms of the anthropometric data, there was a reduction in weight of approx 0.5kg in the metformin group leading to a reduction in BMI of approximately 0.3 kg/m^2. Although these changes did not reach statistical significance in themselves, there was a highly significant difference when the change in weight and BMI were compared between the metformin and placebo groups. There was a reduction in serum leptin within the metformin group but again, the magnitude was not quite large enough to reach statistical significance.

These data are summarised in table 7.12 overleaf. Modest amelioration of insulin resistance was seen with metformin along with some weight reduction and small improvements in some other features of the metabolic syndrome (HDL-cholesterol and trends for blood pressure) as well as some serum markers of endothelial function (tPA and trends for vWF).

Chapter eight goes on to look at the effects of metformin on measures of peripheral microvascular function and clinical measures of ischaemic burden.

Table 7.12 : Summary of changes seen in variables measured in the metformin and placebo groups over the course of the trial (p values shown).

Variable	Metformin n=24	Placebo n=22	Difference between change
fasting glucose	ns	ns	ns
60 min glucose	ns	ns	ns
120 min glucose	ns	ns	ns
fasting insulin	ns	ns	**0.036**
60 min insulin	ns	ns	ns
120 min insulin	**0.019**	ns	ns
fasting quicki index	ns	ns	ns
60 min quicki index	ns	ns	ns
120 min quicki index	ns	ns	ns
fasting NEFA	ns	ns	ns
60 min NEFA	**0.019**	ns	**0.012**
120 min NEFA	ns	ns	ns
Total Cholesterol (mmol/L)	ns	ns	ns
Triglycerides (mmol/L)	ns	ns	ns
LDL-cholesterol (mmol/L)	ns	ns	ns
HDL-cholesterol (mmol/L)	**0.009**	ns	**0.049**
Von Willebrand Factor	ns	**0.036**	ns
Tissue plasminogen activator	**0.001**	ns	**0.045**
D-Dimer	ns	ns	ns
I-CAM	ns	ns	ns
V-CAM	ns	ns	ns
Systolic blood pressure (mmHg)	ns	ns	ns
Diastolic blood pressure (mmHg)	ns	ns	ns
Waist (cm)	ns	ns	ns
Waist : Hip ratio	ns	ns	ns
Weight (kg)	ns	**0.015**	**0.008**
Body Mass Index (kg/m^2)	ns	**0.01**	**0.003**
Leptin	ns	ns	ns
C-reactive protein	ns	ns	ns

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CHAPTER 8

Effects of Metformin on Peripheral Microvascular Function and Clinical Measures of Ischaemic Burden in Women with cardiac 'Syndrome X'

Treatment with Metformin

It has been demonstrated that women with cardiac 'Syndrome X' have impaired peripheral microvascular function compared to healthy controls. Furthermore, in the last chapter the effects of metformin on various metabolic and anthropometric measures were compared to placebo. The rationale for this part of the double-blinded placebo-controlled trial was to determine whether metformin, as an insulin-sensitising modality, would have any effect on:

- Peripheral microvascular function (endothelium-dependent and independent)

- Measures of ischaemic burden
    - Chest pain episodes and GTN use
    - Indices of ischaemia derived from exercise tolerance testing

The theoretical background and the mechanisms of metformin action have been dealt with in chapter 7 along with the tolerability data and the effects on lactate.

Baseline Results

The baseline characteristics of the subjects who completed the trial in both the metformin group (n=24) and the placebo group (n=22) were well-matched at baseline and these data are presented in chapter 7. The only statistical difference was that 4 women in the metformin group were taking calcium channel blockers compared to none within the placebo group, despite attempts to minimise anti-anginal use.

Characteristics of Metformin-treated Group and Placebo-treated Group

Table 1. Clinical characteristics of the metformin and placebo group at baseline.

† mean +/- standard deviation.

	Metformin n=24	Placebo n=22	P value
Chest pain index (episodes/day)	0.45 +/- 0.57†	0.58 +/- 0.91†	NS
GTN use index (usage/day)	0.26 +/- 0.47†	0.06 +/- 0.11†	0.045
Treadmill exercise time (mins)	6.08 +/- 1.55†	6.17 +/- 2.08†	NS
Maximal ST depression (mm)	1.5 +/- 1.2†	1.2 +/- 1.1†	NS
Duke Score	-5.42 +/- 7.6†	-1.86 +/- 6.6†	NS

The above table illustrates that the group randomised to receive metformin were well matched with the group randomised to receive placebo. This is with the exception that the metformin group had a statistically significantly higher frequency of GTN use at baseline. This difference may make the results concerning GTN use after treatment, difficult to interpret.

Presentation of Results

The results for microvascular function and ischaemic measures before and after metformin and placebo are presented in the following pages.
The raw data are shown in a table for both the metformin and placebo groups.

Subsequently, the outcomes are first shown in tabular form with the mean and standard error of mean (SEM) shown at baseline and after the 8 weeks of intervention with either metformin or placebo. The statistical significance of the change is shown next to each set of measurements. Statistical non-significance is represented by 'ns' whereas the p valve for any significant change is shown.

Additionally, the change for each variable, before and after intervention, was calculated for all of the women in both groups, and the difference between this change was looked at. The difference was compared statistically between the groups, using a 2-sample t-test, and this result is shown in the last column of each table. Again 'ns' is simply used to imply that the difference in the mean change between the groups is not statistically significant, with a p valve being presented only when the difference achieved statistical significance.

Finally a boxplot is shown for the important variables measured with the central bar representing the mean and the box representing the standard deviation of the data set. Metformin-treated subjects are displayed adjacent to their placebo-treated counterparts. This is shown to more graphically illustrate the change in variables measured before and after intervention. Bar charts and line charts are used to illustrate any additional data.

1 : Effect of Metformin on LDI Microvascular Function

The raw data are shown in the tables below for the metformin and placebo groups at baseline and then post intervention.

etformin Group at Baseline

Ch-AUC	ACh-AUC (cor for rti*)		SNP-AUC	SNP-AUC (cor for rti*)
5322	18008		3487	11799
961	7200		1228	9200
1128	6830		1598	9676
2032	8766		1019	4396
3243	4809		1378	2043
1265	7825		872	5394
1866	7013		2154	8095
1406	8929		1380	8764
910	3444		1195	4523
1327	8651		1593	10385
4180	26312		1243	7824
1087	8600		928	7342
1767	10885		1322	8144
1544	8760		1539	8732
1061	3630		1484	5077
1106	4026		1260	4586
1848	13229		1487	10645
986	3836		710	2762
817	6364		574	4471
1192	5850		775	3803
1404	7813		884	4919
1185	8479		1260	9016
754	8167		793	8589
1024	10942		912	9745

Metformin Group post intervention

ACh-AUC	ACh-AUC (cor for rti*)		SNP-AUC	SNP-AUC (cor for rti*)
4240	12593		3124	9278
1255	7806		1659	10319
2482	11442		2049	9446
2224	10520		1302	6158
3612	19252		1343	7158
1242	6819		1122	6160
1127	4948		1133	4974
2031	8307		1464	5988
3466	15042		1599	6940
1590	12831		1121	9046
3994	22047		1822	10057
1957	10353		819	4333
1914	19092		1455	14514
1277	8224		695	4476
880	3238		974	3584
2162	13540		1101	6895
2124	18479		1017	8848
1418	9330		938	6172
1082	10084		954	8891
1390	8590		1211	7484
1476	6539		1009	4470
2624	10207		2341	9106
1485	6074		1359	5558
1624	8494		1936	10125

cebo Group at Baseline

Ch-AUC	ACh-AUC (cor for rti*)		SNP-AUC	SNP-AUC (cor for rti*)
1400	11194		954	7628
1105	15135		1028	14080
2586	14264		1340	7391
1388	7123		886	4547
1136	8889		1482	11596
1351	6285		761	3540
1808	7503		2244	9312
1647	8543		1672	8673
1341	14179		1008	10658
526	4651		646	5712
2699	20008		1674	12410
815	5467		692	4642
776	5631		1484	10769
864	4809		940	5232
2462	18926		1541	11846
1359	7366		2749	14845
1212	12666		1004	10492
1051	10362		1370	13507
764	3078		1117	4500
1533	13939		1091	9917
1380	8416		858	5233
973	6568		951	6419

Placebo Group post intervention

ACh-AUC	ACh-AUC (cor for rti*)		SNP-AUC	SNP-AUC (cor for rti*)
2291	12005		1362	7137
1094	13938		1035	13186
2538	12995		1614	8264
1538	8597		1543	8625
1213	9668		895	7133
759	3909		1169	6020
1672	13493		1649	13307
1672	10918		939	6132
1464	7071		1374	6636
1463	7169		1649	8080
1529	11330		1258	9322
1353	10188		871	6559
1036	7107		893	6126
1982	4995		769	1938
2485	18911		1208	9193
1173	5114		1464	6383
2934	16636		2129	12071
734	6980		573	5449
1363	5779		2425	10282
1459	9119		1418	8863
1419	6939		732	3579
857	4928		1113	6400

ti – resistance time integral (see chapter 5)

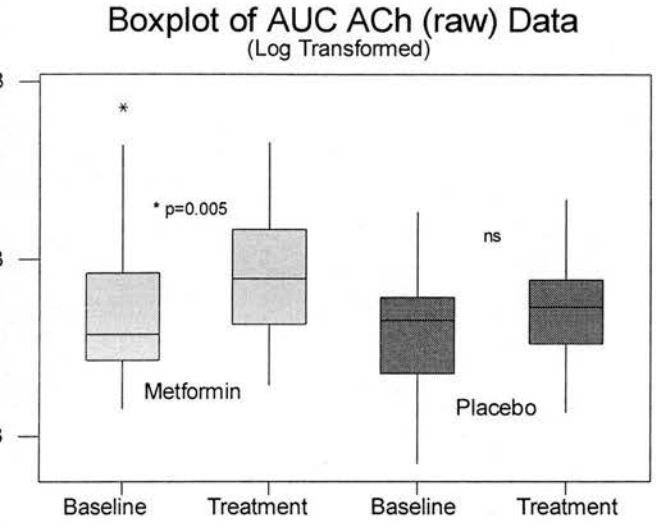
The peripheral microvascular perfusion response data 'area under the curve' (AUC) for ACh and SNP are shown below in table 8.1, both in its raw form and corrected for the skin resistance. These were the 2 most consistent and reproducible ways of representing this data, as demonstrated in chapter 5. Log transformation was required so that the data are normally distributed.

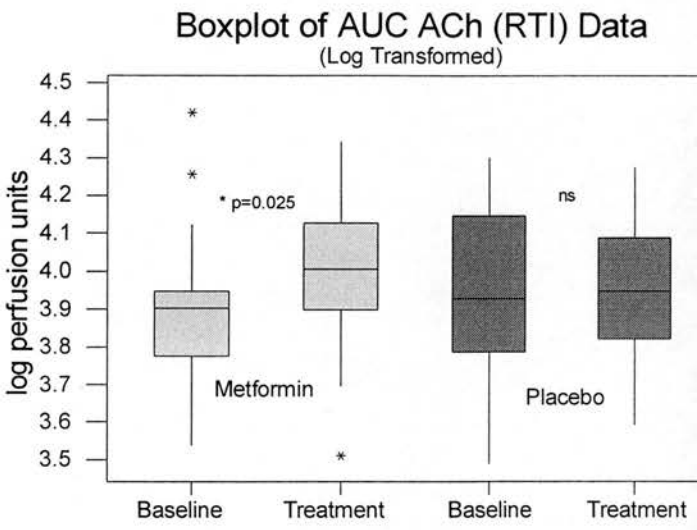
Table 8.1: showing mean change in LDI* microvascular response to ACh and SNP before and after intervention
†*Mean +/- standard error of mean*

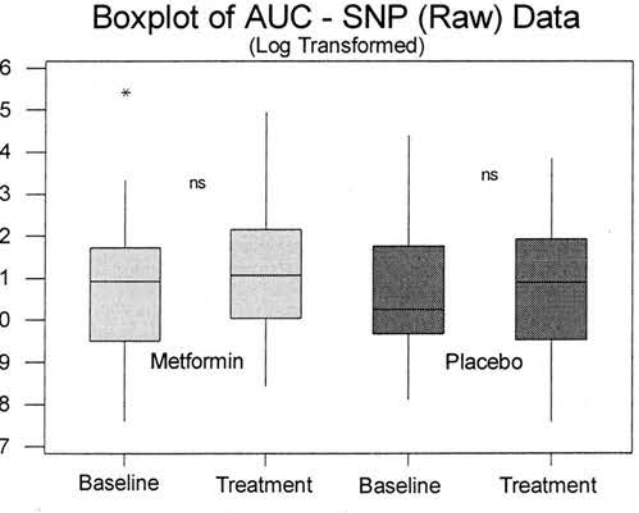
	Metformin group at baseline†	Metformin group after treatment†	p	Mean change	Placebo group at baseline†	Placebo group after treatment†	p	Mean change	Difference between mean change
C ACh (raw) erfusion units)	3.15 +/- 0.044	3.27 +/- 0.038	**0.005**	+0.113+/-0.036	3.10 +/- 0.038	3.16+/- 0.034	ns	+0.059+/-0.040	ns
C ACh (RTI) erfusion units)	3.89 +/- 0.043	4.00 +/- 0.040	**0.025**	+0.113+/-0.047	3.94 +/- 0.045	3.94 +/- 0.039	ns	-0.004+/-0.036	ns
C SNP (raw) erfusion units)	3.07 +/- 0.034	3.12 +/- 0.031	ns	+0.039+/-0.035	3.07 +/- 0.034	3.08 +/- 0.033	ns	+0.013+/-0.046	ns
C SNP (RTI) erfusion units)	3.81 +/- 0.041	3.85 +/- 0.030	ns	+0.040+/-0.043	3.91 +/- 0.040	3.86 +/- 0.039	ns	-0.049+/-0.046	ns

* LDI – laser Doppler imaging

Following the boxplots, there are line charts showing mean ACh and SNP perfusion response for the entire group. Value at each data point is a mean of each data point within the group and Standard Error of the Mean (SEM) is shown by the vertical bars. The values shown are corrected for resistance-time integral as detailed in chapter 5, as this corrects for differences in cutaneous drug delivery and serves to make the results more reproducible.


Boxplot of AUC ACh (raw) Data
(Log Transformed)
* p=0.005
ns
Metformin
Placebo
Baseline
Treatment
Baseline
Treatment


Boxplot of AUC ACh (RTI) Data
(Log Transformed)
log perfusion units
4.5
4.4
4.3
4.2
4.1
4.0
3.9
3.8
3.7
3.6
3.5
* p=0.025
ns
Metformin
Placebo
Baseline
Treatment
Baseline
Treatment


Boxplot of AUC - SNP (Raw) Data
(Log Transformed)
ns
ns
Metformin
Placebo
Baseline
Treatment
Baseline
Treatment

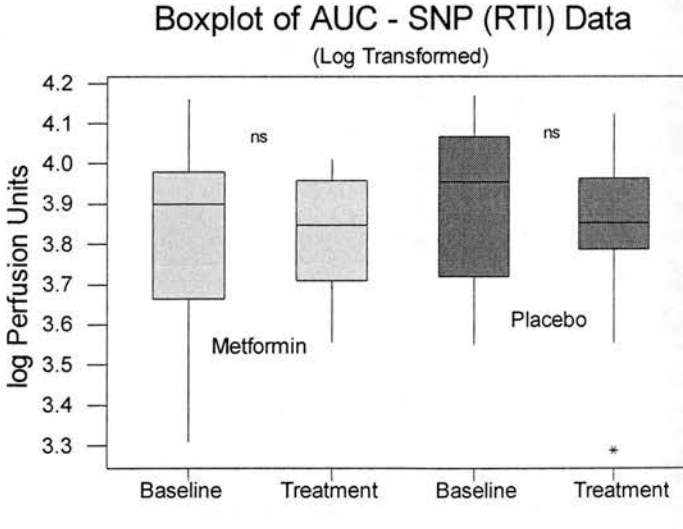

Boxplot of AUC - SNP (RTI) Data
(Log Transformed)
log Perfusion Units
4.2
4.1
4.0
3.9
3.8
3.7
3.6
3.5
3.4
3.3
ns
ns
Metformin
Placebo
Baseline
Treatment
Baseline
Treatment

Figure 1 : Line chart showing mean perfusion response

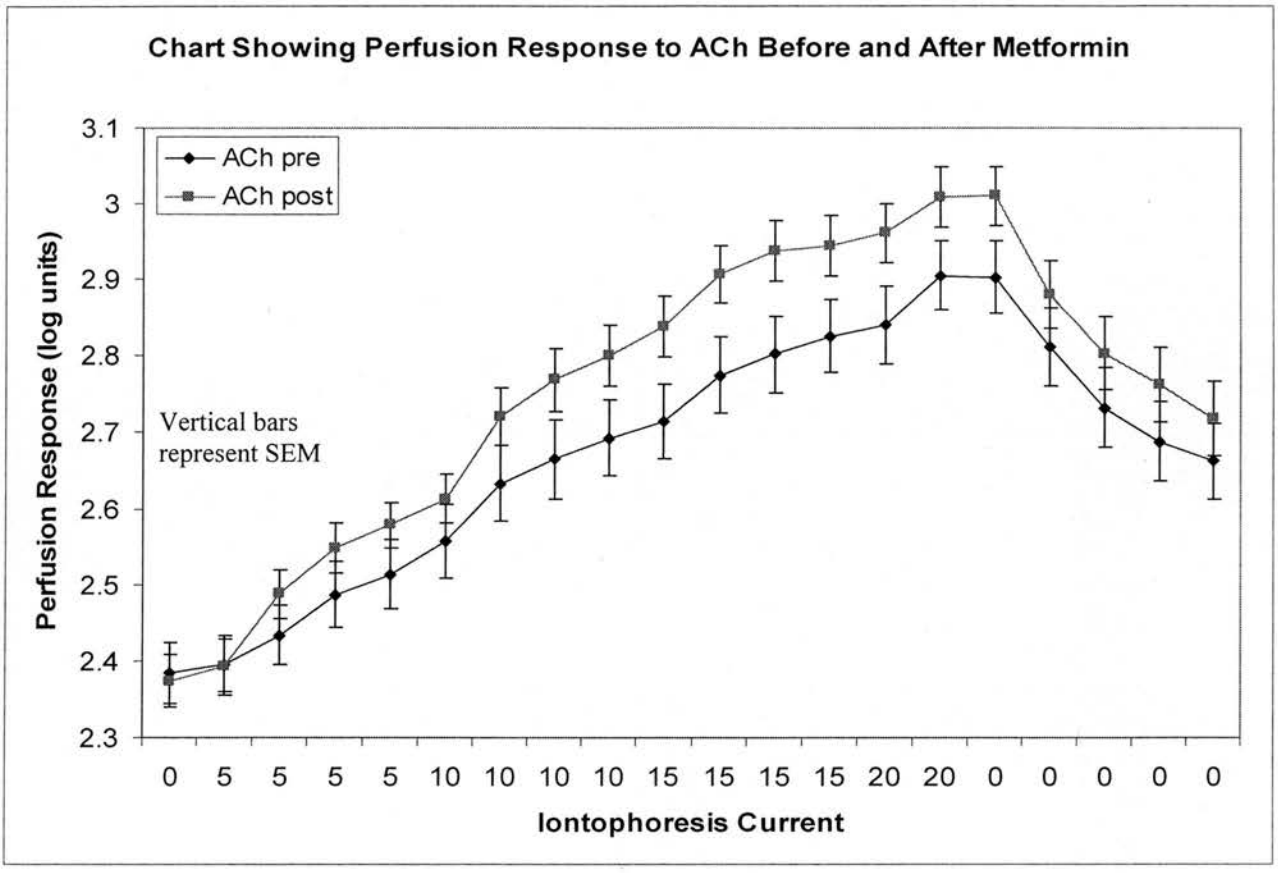


Figure 2 : Line chart showing mean perfusion response

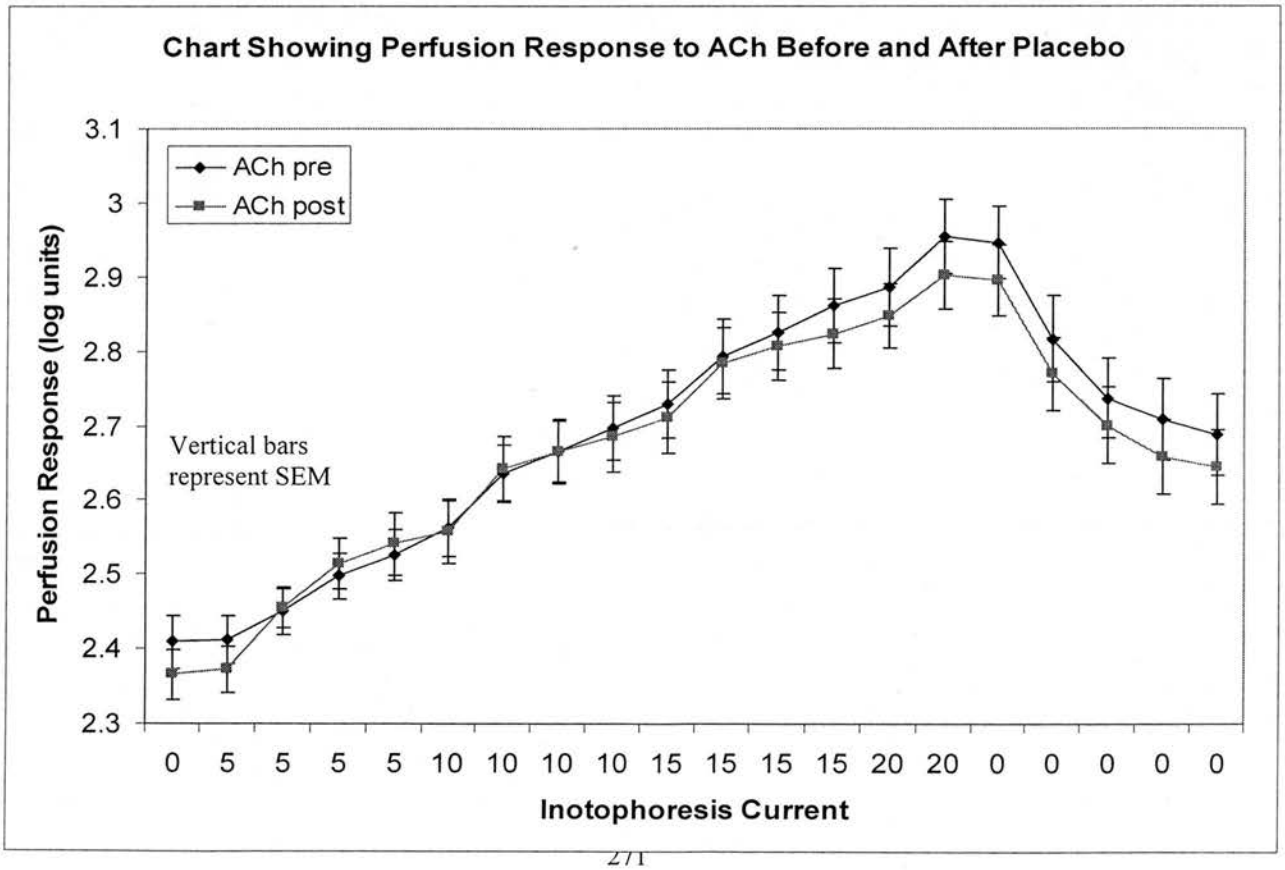


Figure 3 : Line chart showing mean perfusion response

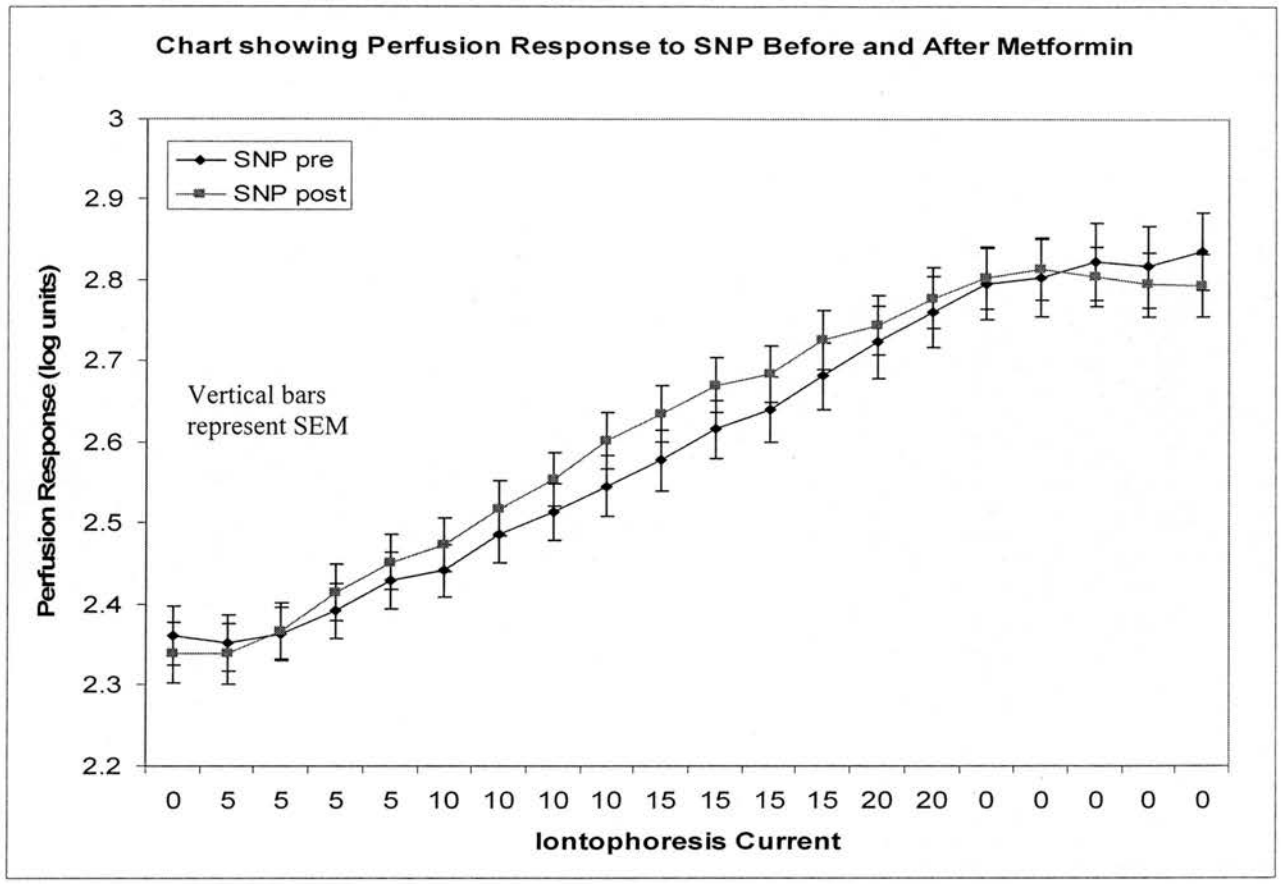
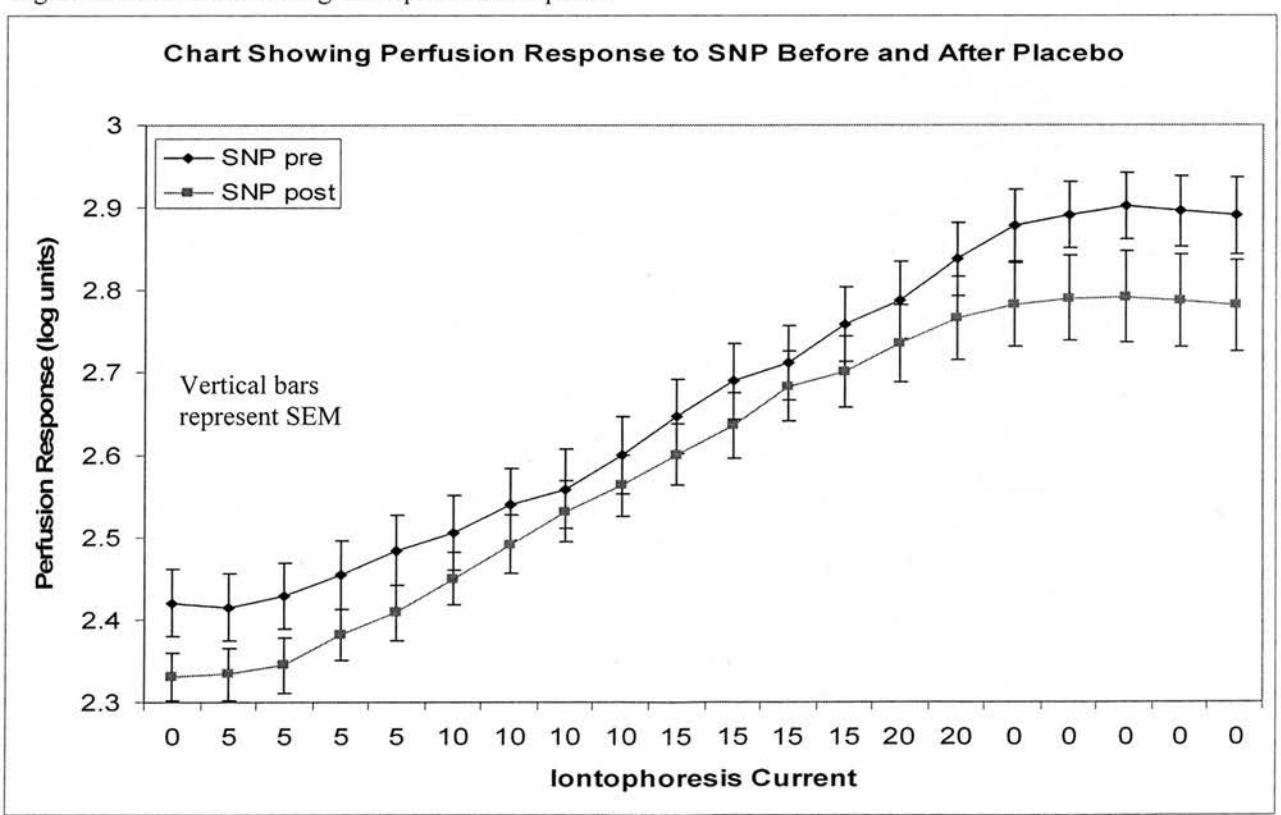


Figure 4 : Line chart showing mean perfusion response



Analysis of Microvascular Function Data

After treatment with metformin for 8 weeks, significant improvements in endothelial-dependent indices of peripheral microvascular function were seen. This is demonstrated by the increased LDI perfusion response to ACh manifested in both the raw data ($p=0.005$) and in the data corrected for the skin resistance ($p=0.025$). The difference before and after treatment was attenuated after correction for skin resistance (as reflected in the higher p value), but none-the-less remained statistically significant.

No difference before and after placebo was observed with a tendency towards improvement seen in the raw data, but virtually no difference after correction for skin resistance ($p=0.924$). These data were presented in chapter 5 where the temporal reproducibility of the laser Doppler imaging technique has been discussed.

The line charts in figures 1 and 2 mirror these results. The lines representing perfusion response to ACh before and after metformin (figure 1) are widely separated and the bars representing the standard error of the mean (SEM) are also separated by the time the current reaches 10mamp, and remain separated until the current is switched off. In contrast, the placebo curves (figure 2) are almost superimposed and certainly there is no trend for the separation of the SEM bars, suggesting no difference in mean perfusion response before and after metformin.

Statistically, no differences emerged in the SNP laser Doppler response after metformin treatment with changes seen comparable to the placebo group. Again, this is borne out by figures 3 and 4. Figure 3 shows the perfusion response to SNP before and after metformin and the curves are almost identical with no separation of the SEM bars. After placebo the curves are more widely apart, but this seems to reflect the difference in the resting perfusion response (the baseline data points in figure 4 are separated). During the phase of the curve when the iontophoresis current is active, the SEM bars are not separated and they are only apart during the phase of the curve at which no iontophoresis current is

applied. This would tend to suggest that metformin had no appreciable effect on the endothelium-independent pathways of microvascular vasodilatation – the vascular smooth muscle response.

This is in agreement with the hypothesis that improvements in insulin resistance will effect improvements in endothelial-dependent vasodilatory capacity via enhanced NO production (amongst other intermediaries). It is interesting to note that the very modest improvements in the indices of insulin resistance were seen in association with improvements in endothelial-dependant microvascular function which were themselves fairly modest.

In summary, improvements in ACh-mediated microvascular vasodilatation were seen after 8 weeks of metformin therapy. No differences were seen in the SNP (endothelium-independent) response.

2 : Effects of Metformin on Measures of Ischaemic Burden

a) Chest Pain Frequency and GTN use

The raw data are shown in the tables below for the metformin and placebo groups at baseline and then post intervention.

tformin pre

est Pain Index	GTN use Index
0.38	0.16
0.88	0
1.14	0
0.107	0.571
0.14	0
0	0
0	0
0.22	0.11
0.83	1.96
0.54	0.54
0.207	0
0.031	0
0.171	0.086
0	0
0.48	0.43
0	0
2.64	1.14
0.25	0.25
0.188	0
0.33	0.148
0.25	0
0.76	0.112
1	0.82
0.312	0

Metformin post

Chest Pain Index	GTN use Index
0.17	0.079
0.52	0
1.61	0
0	0
0.05	0.011
0	0
0.068	0.017
0.18	0.017
0.13	0.2
0.41	0.59
0.057	0.038
0	0
0.056	0
0.097	0.016
0.25	0.25
0.071	0
1.19	0.43
0.14	0.14
0.184	0
0.048	0.032
0.071	0.036
0.75	0.107
0.34	0.0714
0.0635	0

Placebo pre

Chest Pain Index	GTN use Index
0.32	0
0.25	0
0.14	0.11
3	0
0.86	0
3.43	0
0.48	0.48
0.14	0.027
0.89	0.054
0	0
0	0
0	0
0.23	0.17
0.29	0.18
0.86	0
0.071	0.071
0.303	0
0.93	0
0.24	0.12
0	0
0.0588	0
0.2	0

Placebo post

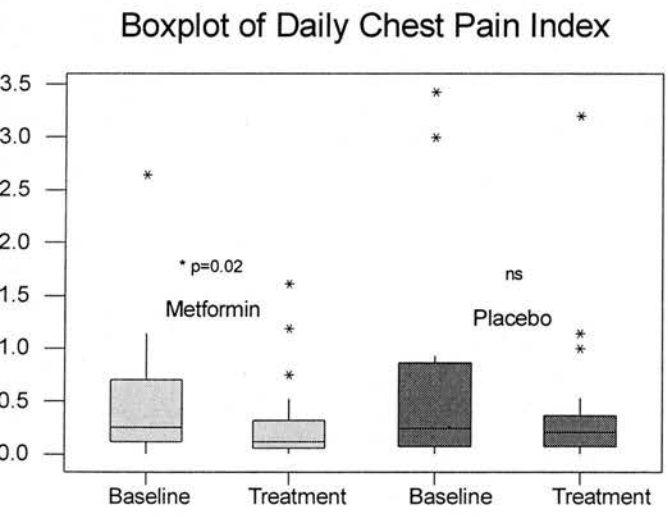
Chest Pain Index	GTN use Index
0.28	
0.13	0.01
0	
1.14	
0.24	
3.2	
0.53	0.5
0.21	
0.35	
0	
0	
0.018	0.01
0.22	0.1
0.35	0.
0.21	
0.054	0.03
0.412	
1	
0.161	0.10
0.21	
0.0794	0.079
0.109	

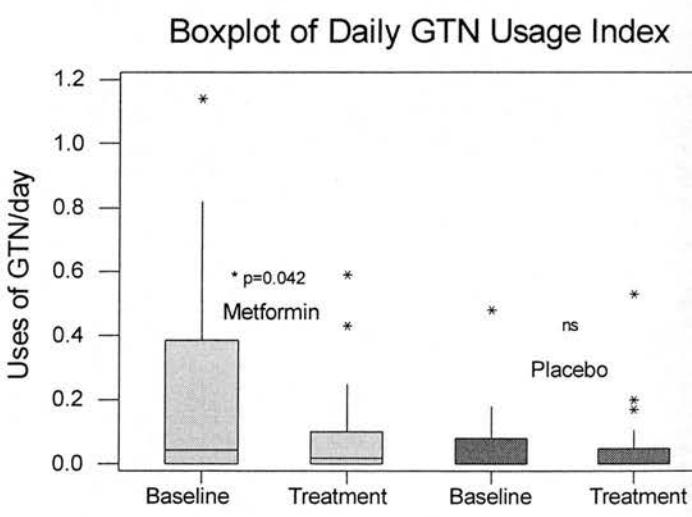
The chest pain index, as discussed in the methods chapter, is a guide to the frequency of chest pain and is essentially the number of episodes of anginal chest pain per day as calculated by the total number of chest pain episodes recorded divided by the number of days over which the diary was kept. A similar index for GTN usage is calculated in the same way. Data are presented below in boxplot form before and after treatment with both metformin and placebo.

The data pertaining to the chest pain index and GTN use for the women in the metformin and placebo groups are summarised below in table 8.2.

Table 8.2: showing mean change in chest pain index and GTN use before and after intervention
†Mean +/- standard error of mean

	Metformin group at baseline†	Metformin group after treatment†	p	Mean change	Placebo group at baseline†	Placebo group after treatment†	p	Mean change	Difference between mean chan
pain Index ɔdes/day)	0.45 +/- 0.12	0.27 +/- 0.08	**0.02**	-0.18 +/- 0.07	0.58 +/- 0.19	0.41 +/- 0.15	ns	-0.17 +/- 0.09	ns
use Index GTN/day)	0.26 +/- 0.10	0.08 +/- 0.30	**0.042**	-0.18+/- 0.08	0.06 +/- 0.02	0.05 +/- 0.03	ns	-0.002 +/- 0.008	**0.045**





b) Treadmill Test Performance

The raw data for total exercise time on the treadmill and maximal ST-segment depression are shown in the tables below for the metformin and placebo groups at baseline and then post intervention.

tformin pre

al exercise time (mins)	ST-segment depression (mm)
6.18	0
4.2	3.5
9.4	0
6.73	2.5
6.72	1
7.35	0.5
2.83	0.5
6.55	3
4.22	2.5
6.73	2.5
6.8	0.5
4.1	0
6.67	2.5
8.48	3
4.35	3.5
6.27	1.5
6.25	1.5
5.75	2.5
6.42	0
8.15	0.5
4.32	1
5.75	2
6.93	1
4.87	0.5

Metformin post

Total exercise time (mins)	ST-segment depression (mm)
4.3	0
2.5	3.5
9.17	0
6.93	2
6.62	2
7.27	0
3.65	0
7.48	1.5
4.32	2
6.85	2
7.38	1
3.68	0
9.02	0
7.77	0.5
5.02	0
4.72	0.5
5.5	1.5
9.35	2.5
6.72	0
9.15	0
4.67	0.5
6.18	1
7.13	0.5
4.05	0.5

Placebo pre

Total exercise time (mins)	ST-segment depression (mm)
3.77	1.5
5.05	0
9.22	0.5
7.25	1.5
9.48	2.5
6	1.5
1.77	0.5
6.33	3.5
7.5	1.5
6.42	3
9.75	0.5
5.88	0
5.5	0.5
6.98	1
6.57	0
8.73	3
4.6	1
2.87	0.5
4.93	0
5.07	3
4.53	1
7.43	0

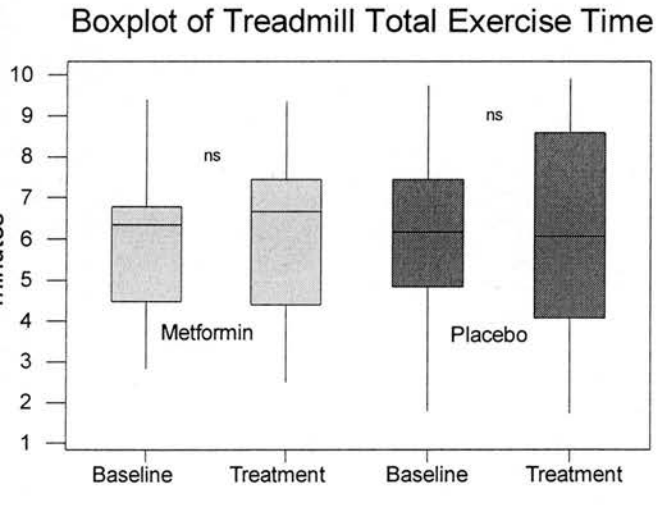
Placebo post

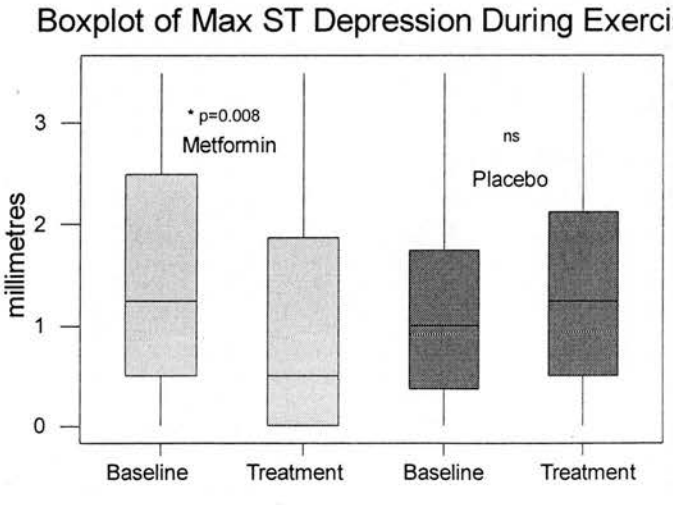
Total exercise time (mins)	ST-segment depression (mm)
3.77	1.5
5.13	0.5
9.22	0.5
8.4	1.5
9.17	3
6.58	1.5
2.22	0.5
6.17	2.5
9.92	0.5
7.47	3.5
9.58	1.5
3.95	0
4.12	0.5
4.78	1
6	0
9.2	3
6.3	1
3.73	1.5
4.8	0
5.05	3
1.72	2
7.5	0.5

All patients underwent treadmill testing before and after treatment with either metformin or placebo. The total treadmill exercise time and the ST-segment depression were the principle measures recorded. These results are summarised below in table 8.3 with boxplots thereafter.

Table 8.3: showing mean change in exercise time and ST-segment depression before and after intervention
†Mean +/- standard error of mean

	Metformin group at baseline†	Metformin group after treatment†	p	Mean change	Placebo group at baseline†	Placebo group after treatment†	p	Mean change	Difference between mean change
exercise time utes)	6.08 +/- 0.32	6.23 +/- 0.40	ns	+0.14 +/- 0.24	6.17 +/- 0.44	6.13 +/- 0.52	ns	-0.04 +/- 0.26	ns
ST-segment ssion (mm)	1.50 +/- 0.24	0.90 +/- 0.20	**0.008**	1.21 +/- 0.24	1.21 +/- 0.24	1.34 +/- 0.23	ns	+0.14 +/- 0.11	**0.003**





c) Duke Score

As described in the methods section, the Duke Score is a widely used scoring method which is used to reflect the overall treadmill performance. It has been shown that the Duke Score is correlated to mortality in patients with obstructive coronary disease.

These boxplots represent the Duke Score data. The Index is derived from :

- 0 – no chest pan on treadmill
- 1 – non-limiting chest pain on the treadmill
- 2 – Chest pain which limits exercise

The Duke score which reflects overall treadmill performance is then calculated by:
(Exercise time in mins) – (5 x Max ST-segment deviation) – (4 x index)

Raw data for the Duke Score are shown below for both groups

nin pre *Metformin post* *Placebo pre* *Placebo post*

x	Duke Score		Index	Duke Score
2	-1.82		0	4.3
1	-17.3		1	-19
1	5.4		0	9.17
0	-5.77		0	-3.07
1	-2.28		1	-7.38
0	4.85		0	7.27
1	-3.67		0	3.65
1	-12.45		1	-4.02
1	-12.28		0	-5.68
2	-13.77		2	-11.15
0	4.3		1	-1.62
1	0.1		0	3.68
1	-9.83		0	9.02
1	-10.52		0	5.27
2	-21.15		1	1.02
1	-5.23		1	-1.78
2	-9.25		0	-2
1	-10.75		1	-7.15
0	6.42		1	2.72
0	5.65		0	9.15
2	-8.68		1	-1.83
1	-8.25		1	-2.82
1	-2.07		1	0.63
1	-1.63		1	-2.45

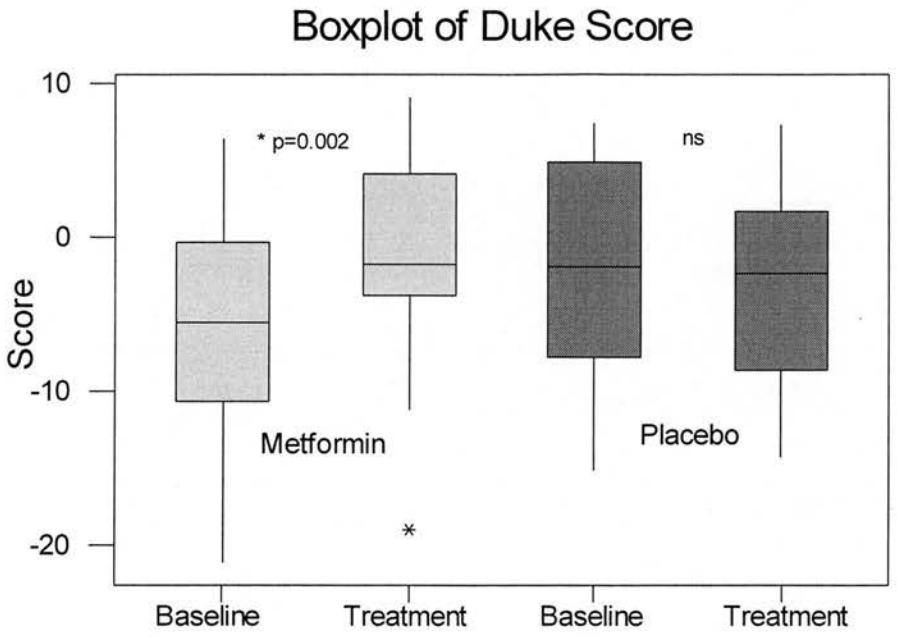
Index	Duke Score		Index	Duke Score
1	-7.73		2	-11.73
0	5.05		1	-1.37
1	2.72		1	2.72
0	-0.25		0	0.9
0	-3.02		0	-5.83
1	-5.5		1	-4.92
0	-0.73		0	-0.28
1	-15.17		2	-14.33
2	-8		0	7.42
0	-8.58		1	-14.03
0	7.25		0	2.08
0	5.88		0	3.95
0	3		0	1.62
2	-6.02		2	-8.22
0	6.57		2	-2
0	-6.27		0	-5.8
1	-4.4		1	-2.7
0	0.37		1	-7.77
0	4.93		0	4.8
0	-9.93		0	-9.95
2	-8.47		1	-12.28
0	7.43		1	1

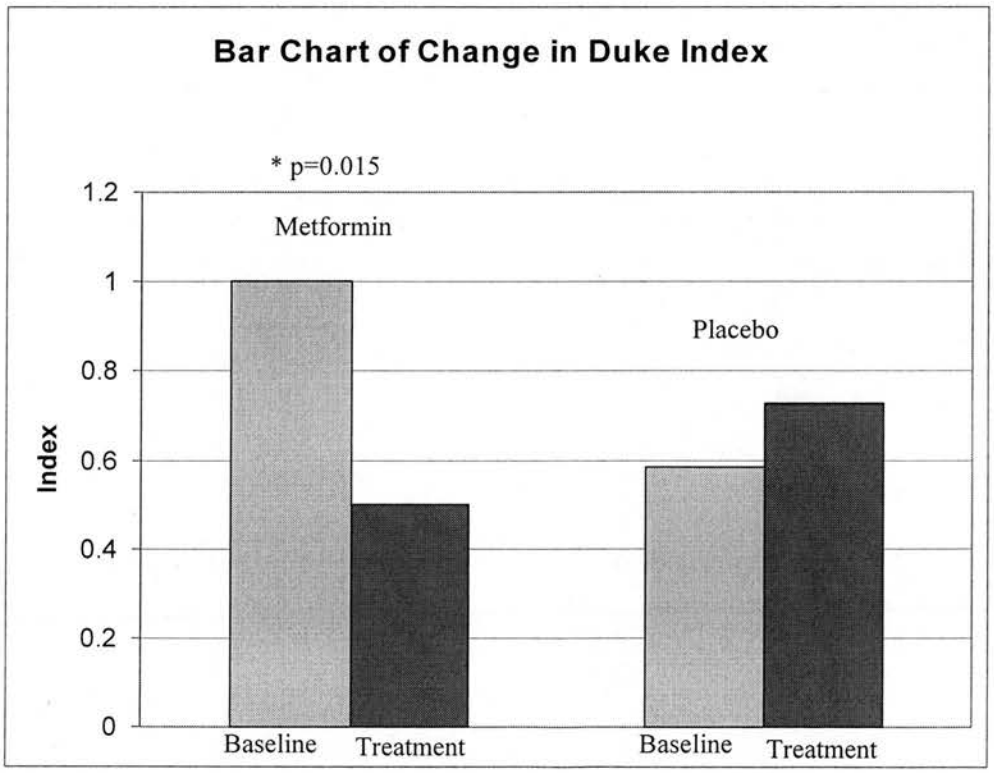
This generally gives a score of between -18 and 12 with a more positive score correlating with a better overall treadmill performance.

The data pertaining to the Duke Score for the women in the metformin and placebo groups are summarised below in table 8.4 and thereafter represented in the boxplots and bar charts below.

Table 8.4: showing mean change in Duke Score index and total Duke Score before and after intervention
†*Mean +/- standard error of mean*

	Metformin group at baseline†	Metformin group after treatment†	p	Mean change	Placebo group at baseline†	Placebo group after treatment†	p	Mean change	Difference between mean chang
Score Index	1.0 +/- 0.14	0.58 +/- 0.12	**0.015**	-0.42 +/- 0.16	0.50 +/- 0.16	0.73 +/- 0.16	ns	+0.23 +/- 0.17	**0.009**
Duke Score	-5.42 +/- 1.55	-0.59 +/- 1.38	**0.002**	+4.48 +/- 1.37	-1.86 +/- 1.40	-3.49 +/- 1.37	ns	-1.63 +/- 1.05	**0.001**


Boxplot of Duke Score
10
0
-10
-20
Score
* p=0.002
ns
Metformin
Placebo
*
Baseline
Treatment
Baseline
Treatment


Bar Chart of Change in Duke Index
* p=0.015
Metformin
Placebo
1.2
1
0.8
0.6
0.4
0.2
0
Index
Baseline
Treatment
Baseline
Treatment

Analysis of Angina/Treadmill Data

Significant changes are seen in the episodes of chest pain and GTN-use logged in the patients diaries before and during treatment with metformin but not with placebo. However a downward trend in the frequency of chest pain with placebo also occurs, just missing out on statistical significance. This could mean at least part of the reduction in chest pain frequency that occurred with metformin is due to increased medical contact and hospital visits during the study. GTN-use also exhibited a downward trend in the placebo-treated group and the statistical significance achieved in the metformin-treated group may be in part due to a higher baseline GTN-usage in this group (0.26 Vs 0.05 equating to a difference of GTN use once every 4 days in the metformin group compared to once every 20 days in the placebo group). The change seen in the metformin group could be seen as simply regression to the mean, and the baseline differences between the groups from this respect make the changes difficult to interpret.

The above measures were recorded in patient diary charts but none-the-less are fairly subjective. More objective measures in symptoms and indices of ongoing myocardial ischaemia are obtained from the treadmill test results. Although a minor increase in exercise capacity is seen in the metformin-treated group (an average increase from 6'05 to 6'13) this was insufficient to obtain statistical significance. However, this compares with a small fall on average in exercise capacity in the placebo-treated group. The index based on chest pain during the test improved significantly in the metformin group but deteriorated non-significantly in the placebo group. This also provides some measure of amelioration in overall anginal symptoms.

ST-segment depression during exercise fell significantly after treatment with metformin from an average of 1.5mm at baseline to less than 0.9mm after treatment ($p=0.008$). This compares with a small increase on average in ST depression in the placebo-treated group from approximately 1.2mm to 1.3mm. There were also significant changes in the Duke Score after metformin treatment with an average increase in the score of over 5 ($p=0.002$)

compared to the placebo group whose average Duke score fell by just over 1.5 but was not significantly different from baseline.

In summary, there are weak subjective improvements with metformin in anginal symptoms and GTN-use compared to placebo. More objective benefits on non-invasive markers of myocardial ischaemia were also seen with improvements in ST-segment depression and Duke Score during treadmill testing. The objective improvement in the chest pain index during the ETT with metformin, reinforce the improvement in overall symptoms. However, although trends towards slightly improved exercise capacity after metformin emerged, these were statistically insignificant.

CHAPTER 9

Summary and Discussion

Insulin Resistance – Measurement, Aetiology and Link to Cardiovascular Disease

Insulin sensitivity is a physiological concept which essentially describes how efficiently whole body insulin-medicated glucose uptake occurs in an individual. Insulin sensitivity varies widely in populations along a spectrum. 'Insulin Resistance' is the term used to describe individuals at one end of this spectrum where insulin-mediated glucose uptake is particularly inefficient. Although it is not accurate to describe these individuals as having 'abnormal' insulin sensitivity, there is now good evidence that metabolism weighted in this direction does have significant clinical repercussions.

Several well-established cardiovascular risk factors cluster around 'insulin resistance' – including hypertension, dyslipidaemia, and central adiposity but even correcting for these factors, there is now strong prospective evidence that hyperinsulinaemia, as a marker for insulin resistance, confers at least a modest independent cardiovascular risk. This seems to be mediated by accelerated atherogenesis and there are compelling data demonstrating increased atheroma loads with relative insulin resistance, both in the coronary circulation and more generally as manifest by ultrasound studies of the carotid arterial system.

The 'gold-standard' method for assessing insulin resistance is by actually measuring insulin-mediated glucose uptake under hyperinsulinaemic conditions – the so called hyperinsulinaemic euglycaemic clamp technique. This is a very cumbersome, invasive and time-consuming method but there are data showing that less invasive methods based around measuring serum insulin and/or glucose under fasting conditions or post glucose load, correlate to some degree with clamp-derived indices of insulin resistance. Specifically, the 'quicki index' which is based on both serum insulin and glucose and which incorporates a logarithmic and reciprocal transformation in its calculation has been shown to have good correlations to the clamp technique especially in the obese, insulin-resistant populations studied.

Insulin signalling involves a complex intracellular network which is initiated by self-phosphorylation of the insulin receptor after binding to insulin. This produces in turn activation of phosphatidylinositol 3-kinase (PI 3-kinase) which appears to be a central mediator of the intracellular effects of insulin. This intracellular cascade (which involves some elements independent of PI-3 kinase also) culminates in the translocation of

GLUT4 to the cell membrane which facilitates the transport of glucose across the cell membrane (see chapter 1, figure 4). There are other intracellular roles of insulin which are less well-understood, such as the effect on vascular tone and it seems that PI-3 kinase has a crucial role in these also.

The aetiology of insulin resistance is multi-factorial in most cases although extreme forms have been described in association with discrete mutations affecting part of the post-receptor signalling cascade. There are several different mechanisms by which insulin resistance may be promoted with genetic influences forming a background on which environmental factors such as body fat distribution and systemic inflammation can interact. However, it is clear that these influences do not act in isolation and it is likely that they work together, with even the possibility of positive feed-back and amplification once the processes to promote insulin resistance have been initiated.

Cardiac Syndrome X –

Myocardial Ischaemia, Insulin Resistance and Vascular Dysfunction

It is known that vasomotor disturbances are present in atheromatous coronary arteries and it is likely that this phenomenon contributes to myocardial ischaemia in patients with obstructive disease. Patients with 'Syndrome X' have no obstructive coronary atheroma and yet there is strong evidence that at least a subgroup of these patients have myocardial ischaemia, as manifest by abnormalities in myocardial lactate metabolism (see table 2, chapter 2) under stressor conditions.

There is a wealth of data demonstrating impaired coronary vascular responses under stress, in this population with normal coronary arteries angiographically (see table 3, chapter 2) and this is potentially the mechanism producing myocardial ischaemia in these patients. Furthermore, there is evidence to show that there is a generalised impairment in vascular function, which is not confined to the coronary vascular bed.

The weight of evidence in the literature shows that patients with 'Syndrome X' are insulin-resistant compared to healthy controls and this may be the mechanism leading to impaired vasodilator capacity. The vasodilating effects of insulin are well-described and specifically, it is known that nitric oxide (NO) generation by the enzyme nitric oxide synthase is coupled to insulin signalling. Other factors such as abnormalities of endothelin-1 (a potent vasoconstrictor) and relative oestrogen deficiency (which also has vascular effects) may also contribute to the abnormal vascular responses seen in patients with 'Syndrome X'.

Groups who have studied patients with 'Syndrome X' have reported a female predominance and a link to relative insulin resistance along with some other features of the metabolic syndrome. This pattern fits with the potential aetiological mechanisms discussed. Although these patients have angiographically unobstructed coronary arteries, there is evidence that many have sub-angiographic atheromatous coronary plaques.

It is becoming clear that the prognosis in this group is not as benign as previously thought. The burden of morbidity related to ongoing symptoms, recurrent hospital admissions and even repeat coronary angiography is very significant. There are also

reports of these patients sustaining myocardial infarction and cardiac death, although the frequency of these adverse events are much lower than the group of patients with obstructive cardiac disease.

Metformin in Insulin Resistance and Syndrome X (MIRS) Study-
Trial Design and Methods

This was a randomised double-blind placebo controlled trial designed to examine the difference in women with 'Syndrome X' and healthy controls at baseline. The effects of 8 weeks of metformin on metabolic, anthropometric, vascular and ischaemic measures were assessed compared to placebo.

Female patients were recruited on the basis of a normal coronary angiogram between 1998-2001 and there were 71 subjects who fulfilled the entry requirements of this trial in having a documented exercise tolerance test showing at least 1mm of flat ST-segment depression. The main exclusion criteria were diabetes, uncontrolled hypertension, structural heart disease and significant renal or hepatic impairment. Of the 67 eligible women, 59 agreed to initial assessment for this trial and 56 were enrolled into the study ultimately. Anti-anginals were minimised and in most cases stopped for 4 weeks prior to the study.

Patients and controls attended in a fasting state for blood tests (oral glucose tolerance test in 'Syndrome X' patients only), peripheral microvascular assessment, clinical measures of blood pressure and body mass index. Patients with 'Syndrome X' also had detailed assessment of their chest pain and nitrate use, and underwent a standard exercise tolerance test (full Bruce protocol) and echocardiography.

The 'Syndrome X' group were randomised to either metformin 500mg BD (n=27) or placebo (n=29) for 8 weeks and then had all the investigations repeated to ascertain whether this 8 week period of treatment had any impact on the metabolic, vascular or ischaemic measures. Details of the study protocol and methods used are within chapter 3.

Assessing Peripheral Microvascular Response

There are several techniques available for looking at vascular function both in the coronary circulation and peripherally. These vary in the size of vessel looked at and the degree of invasiveness associated (see table 1, chapter 5). Laser Doppler imaging was used in this trial to assess peripheral microvascular vasodilation in response to iontophoresis of acetylcholine (ACh) and sodium nitroprusside (SNP). This has the advantage of being completely non-invasive and is specifically assessing the microcirculation as opposed to larger vessel responses which are looked at by other techniques.

The principles behind this technique and the specifics of the protocol used are discussed in detail within chapter 5, but briefly, this technique uses the Doppler shift in the wavelength of a reflected laser beam to calculate the change in cutaneous perfusion during iontophoretic application of vasoactive drugs.

The reproducibility of this technique was looked at with repeat scans between arms and repeat scans after 8 weeks. There are several methods of analysing the results, including correction for skin resistance (which alters the delivery of drugs by iontophoresis) and correcting for the baseline value of perfusion (which likely reflects baseline microvascular tone). It was shown that the good results in terms of reproducibility are obtained by using the raw value of perfusion but this is improved slightly by correcting for skin resistance (method described in chapter 5). Correcting for the baseline perfusion unit does not appear to be a useful exercise in promoting reproducibility of the results and this suggests that baseline microvascular tone may be of some importance.

Inter-arm mean variation for the AUC response (area under the curve) was 2.7% for ACh and 3.8% for SNP. Temporal variation (scans 8 weeks apart) had 3.5% average variation for ACh and 4.7% for SNP. There are many factors which influence the perfusion response obtained by laser Doppler imaging in conjunction with iontophoresis and some are beyond control (such as emotional state of the patient). However, these data provide some reassurance in that as long as a standardised protocol is performed under controlled conditions, results with good reproducibility are obtained.

Assessment at Baseline –

Comparisons Between Healthy Controls and Women with 'Syndrome X'

The 56 women in the 'Syndrome X' group were on average 5.5 years older and had a body mass index (BMI) 3.5 m^2/kg higher than the 25 healthy controls. Waist measurements were also approximately 7cm lower in the controls group.

There were no significant differences between the groups in smoking history nor family history of ischaemic heart disease or diabetes. During the trial period, anti-anginals were minimised in the 'Syndrome X' group to such an extent that there were no differences in the use of beta-blocker, calcium channel blockers, nicroandil or nitrate preparations, to that of the control group. There was much greater use of aspirin and statins amongst the 'Syndrome X' group during the trial.

Women with 'Syndrome X' showed higher fasting serum insulin and a lower fasting quicki index suggesting relative insulin resistance compared to controls. This was associated with some other features of the metabolic syndrome compared to controls in terms of higher systolic blood pressure (14mmHg on average) and dyslipidaemia (triglycerides higher in 'Syndrome X' group). There were higher levels of serum endothelial markers namely, von Willebrand factor (vWF), tissue plasminogen activator (tPA), intracellular adhesion molecule (I-CAM) and vascular cell adhesion molecule (V-CAM). High sensitivity C-reactive protein and serum leptin were higher in the 'Syndrome X' group reflecting as markers for inflammation and adiposity respectively.

However, once the difference in age and BMI between the groups were addressed, differences between the groups remained only for fasting insulin and quicki index, triglycerides, HDL-cholesterol, vWF and I-CAM, and serum leptin. These results are summarised in table 4.1 and 4.2 within chapter 4.

Looking at microvascular function, there was significantly increased microvascular vasodilation with ACh and SNP in healthy controls compared to women with 'Syndrome X'. These highly significant differences persisted even after correction for the age and BMI differences between the 2 groups. This suggests that as well as a blunted endothelium-dependent response, there also exists impairment in the endothelium-

independent response which points to more generalised vascular dysfunction, perhaps involving the vascular smooth muscle.

Weak associations between the quicki index of insulin sensitivity and microvascular function were seen in all of the women – 'Syndrome X and healthy controls - (r^2=7% for endothelium-dependent and 6% for endothelium-independent responses). This is compatible with the link between insulin sensitivity and vascular function which has previously been described.

These data are important and in some respects unique in that I have now extended the spectrum of risk factor abnormalities in women with Cardiac Syndrome X to also include vWF, ICAM-1, leptin and potentially t-PA and CRP. The elevation in leptin is of particular interest since a recent report has linked leptin to vascular dysfunction independently of other pathways (1). Moreover, there is plentiful evidence that inflammatory pathways are relevant to vascular dysfunction (2). These observations are relevant since many of these parameters are recent additions to the spectrum of perturbances linked to the metabolic syndrome, and thus, in turn, to insulin resistance

Correlations between vasomotor dysfunction in the peripheral and coronary vascular beds have been documented (3). If attenuated peripheral microvascular function serves as a marker for coronary microvascular dysfunction then it may be possible to identify those with coronary microvascular dysfunction non-invasively, on the basis of their peripheral microvascular function. This has important implications for the potential identification of patients with 'microvascular angina' within the group of 'Syndrome X', as ECG criteria are often unhelpful and can be difficult to interpret.

In conclusion, I have made several novel and potentially clinically important observations in this study. I have extended the spectrum of metabolic and inflammatory derangements evident in women with Cardiac Syndrome X and shown that they have markedly impaired skin microvascular function as assessed by laser Doppler imaging, suggesting the presence of generalised vascular dysfunction. Future prospective studies are required to address whether this simple non-invasive technique has clinical utility in diagnosing this syndrome.

Effects of Metformin on Metabolic Parameters, Vascular Function and Ischaemic Measures in Women with Cardiac 'Syndrome X'

I showed that metformin is relatively well-tolerated and in fact there was only an 11% drop out rate for metformin compared to 24% with placebo. There were modest increases in serum lactate during the 8 weeks of the trial both in the metformin and placebo groups. The reason for the rise in the placebo group is not apparent, but it does lead to the suggestion that the increase in lactate seen with metformin may not be due to metformin treatment per se.

The women in the metformin group (n=24) were well-matched with the placebo group (n=22) in terms of smoking, menopause and drugs use. There was a small excess in the number of women taking calcium channel blockers in the metformin group. Both groups were well-matched in terms of their baseline metabolic and anthropometric variables. Looking at ischaemic measures, GTN use was greater in the metformin group at baseline compared with the placebo group.

Regarding indices of insulin resistance, there was a small but significant reduction in 120min post-glucose serum insulin after metformin treatment. There was also a significant difference between the change in fasting insulin between the metformin and placebo group in favour of lower serum insulin with metformin. With the other features of the metabolic syndrome in mind, HDL-cholesterol rose significantly post metformin treatment but there was no significant change in blood pressure. Considering the serum endothelial markers, only tPA fell significantly post metformin with no change in vWF, D-dimer, I-CAM nor V-CAM. Metformin effected a net weight loss and subsequent reduction in body mass index compared to the changes seen with placebo, but there was no change in waist measurement, nor any statistically significant change in C-reactive protein nor leptin. These data are summarised in table 12, chapter 7.

The microvascular function data showed improvement in the ACh (endothelium-dependent) response after metformin but no significant change after placebo. No change was seen in the SNP response after metformin or placebo. This suggests that the modest improvement in insulin sensitivity with metformin effected favourable changes in the

endothelium-dependent microvascular system, in line with the known vascular effects of insulin.

These metabolic and vascular changes did have some impact on clinical measures of ischaemic burden. There were significant reductions both in the recorded frequency of chest pain and GTN use in metformin-treated patients. Although there was no significant increase in treadmill exercise time, there was much improved ST-segment change with metformin and a much improved Duke score suggesting improved overall treadmill performance.

As far as I am aware this is the first placebo-controlled study to show that metformin improves endothelial-dependent function in a non-diabetic patient group. Since abnormal endothelial function may be causally linked to both diabetes (4;5) and CHD (6;7), these findings are significant and concur with increasing suggestive evidence for vascular risk reduction with metformin in type 2 diabetes (8-10) and more robust data for prevention of type 2 diabetes in subjects with impaired glucose tolerance (11). This controlled study also provides tantalising support for a metformin-induced reduction in myocardial ischaemia in women with exercise-induced angina but normal coronary arteries. The noted beneficial effects of metformin on vascular function, metabolic parameters and ischaemia suggest that this drug may have the potential to not only improve clinical symptoms but also to lessen vascular risk in the longer term. These findings are important since a large number of patients worldwide, particularly women, may have this condition and its prognosis is not as benign as previously considered (12).

Why should vascular function improve with metformin? If insulin resistance is a precursor to endothelial dysfunction, it follows that ameliorating insulin resistance might improve it. In fact, there was a significant reduction in tPA concentration (partially endothelial derived) and it is notable that elevated t-PA has recently been shown to be an independent predictor of both CHD events (13) and type 2 diabetes (14). These results suggested that improvements in directly-measured vascular function could not be accounted for by glucose or inflammation parameters, which did not alter in our non-diabetic population of women. Thus, the findings confirm and extend work of De Jager and colleagues who reported beneficial effects of metformin on circulating markers of endothelial function were independent of glycaemic changes in patients with type 2

diabetes (15). It should be noted that metformin has other complex intra-cellular actions, one of which is activation of adenosine mono-phosphate kinase (AMP-kinase) (16) , which in turn has been shown to directly activate endothelial nitric oxide synthase (17;18). This mechanism in itself may contribute to the vasoactive effects of metformin independently of its action on insulin action.

This study was powered to detect change in endothelial function but I was also interested in examining any potential effects of metformin in reducing ischaemia given prior - albeit uncontrolled - evidence of such benefits with metformin and troglitazone in patients with diabetes and angina (reviewed in (19)). These data suggest that metformin may lessen ischaemic burden as determined by reduction in maximal ST depression, Duke score and episodes of chest pain. Again, the inclusion of a placebo group, which demonstrated minimal alterations in any of these parameters, serves to strengthen the robustness of these findings. Although other anti-anginals were stopped prior to this study, these findings are important and suggest that metabolic and vascular function manipulation might be a novel route to lessen myocardial ischaemia. Of interest, other current agents being tested in ischaemic heart disease (e.g. trimetazidine) target metabolic manipulation to improve symptoms and appear to be insulin sensitising (20). In addition, there is now emerging evidence that the potent insulin sensitisers, peroxisome proliferator-activated receptor-gamma (PPARγ) activators regulate activity of endothelial NO synthase (21), lessen anginal pain (22) and improve myocardial blood flow (23).

In summary, using a double-blind placebo controlled study, I have shown that metformin improves endothelium-dependent vascular function, vascular risk parameters, and ischaemic burden in women with a history of exercise-induced angina but normal coronary arteries on angiography. Such effects suggest that metformin may have the potential not only to improve clinical symptoms in this patient group, but also to lessen their vascular risk in the longer term. In addition, it may have preventive and therapeutic applications in other non-diabetic patient groups. Larger controlled studies are now required to expand these novel findings.

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Consent Sheet

Greater Glasgow Health Board

Glasgow Royal Infirmary - Research Ethics Committee Patient Consent Form

Brief Title of Project: The effects of Metformin in women with angina but who have normal appearance of the blood vessels of the heart.

Patients Summary

I understand that a team of researchers under the supervision of Professors Cobbe and Greer and Dr Sattar are investigating the effects of the drug Metformin on chest pain incidence and exercise capacity in women with angina but normal coronary blood vessels on angiography.

The study involves the following:

1. If possible reducing the current 'cardiac' medications to a minimum for the duration of the study (12 weeks)
2. Having a series of investigations performed 4 weeks later including :
   - Fasting overnight followed by a sugar drink and blood sampling in the morning.
   - Measurement of the skin blood flow by LASER Doppler technology (this technique is safe and painless and does not involve further blood sampling or invasive procedures)
   - A detailed exercise test on a treadmill
   - An ultrasound scan of the heart (Echocardiogram)
   - Recordings of blood pressure and heart rhythm
3. Thereafter, I will be randomised to receive either the drug Metformin (often used to treat patients with diabetes) or matching placebo for a total of 8 weeks.
4. All the above investigations will be repeated in 8 weeks time apart from the echocardiogram.

Giving a blood sample may be associated with minor discomfort. I understand that my involvement in the study is entirely voluntary and that I may withdraw at any time without any reason being given, and without my medical care and legal rights being affected. I also understand that the study may be of little benefit to me but the results may help other patients in the future.

CONSENT

I, (Name) _____

Of (Address) _____

_____

agree to take part in the Research Project Study Programme described above.

Dr/Mr _____ **has explained to me the nature of my involvement, how it may affect me and the purpose of the Research Project/Study Programme.**

Signed _____ **Date:** _____

Witness _____ **Date:** _____

Information Sheet on Study – The Effects of Metformin in Women with Cardiac Syndrome X

Thank you for taking the time to read this information sheet in which we invite you to be involved in a clinical study at Glasgow Royal Infirmary.

Background

Cardiac syndrome X refers to individuals who experience frequent episodes of chest pain like angina, but who have a normal appearance of blood vessels around the heart. The condition is more common in women, often beginning around the change of life (menopause), and may be related to an increased susceptibility to diabetes. Treatment of women with this condition is difficult, although some success has been noted with hormone replacement therapy (HRT). However, many women find HRT unsuitable, so there is a need to develop other treatments. In this study, which is funded by the British Heart Foundation, we plan to examine if the drug 'Metformin', commonly used to treat diabetes, can help to reduce the frequency of chest pain in women with syndrome x.

What would your involvement entail ?

You have been asked because you have angina but normal-looking blood vessels around the heart. If you would like to be involved, we will invite to the Royal Infirmary to ask you some questions and examine you. If possible we will take you off all your cardiac medications for 4 weeks prior to, and for the duration of the study (a total of 12 weeks). If this is not possible, you will be eligible for the study so long as you remain on the same medications throughout the study. We will then perform a series of investigations as listed below (1-5). Following these tests you will be randomised to receive either Metformin at a dose of500mg twice daily or matching placebo (a dummy tablet which has no clinical effect), for a total of 8 weeks. The identity of the tablet you receive will not be known to the researchers or yourself. Some of the investigations below (1-4) will be repeated in week 8 of your treatment. Apart from minor stomach upset often in the first week, Metformin is generally free from side-effects. If the trial is successful, it is possible that Metformin may be used in future to treat women with cardiac syndrome x.

Investigations to be performed

Your involvement would entail 5 different procedures:

1. Fasting overnight followed by a sugar drink and blood samples in the morning
2. Measurement of skin blood flow which we assess with LASER techniques along with topical application of some chemicals. This particular technique is entirely safe and painless and takes around one hour to complete.
3. We will put a heart rate monitor on you for 2 hours in the morning and measure your blood pressure 3 times.
4. We will exercise you on a treadmill and measure your exercise capacity along with other measures normally carried out with this procedure
5. We will perform an ultrasound scan of your heart (Echocardiogram). This involves applying jelly to the front of the chest and scanning with a probe. It is entirely painless.

At the end of the study, if appropriate, we will put you back on all your previous medications and your clinical care will continue as before. We will make arrangements for you to be seen at the cardiology clinic for follow-up. The results will be published in a scientific journal.

If you require further information, please ask. If you are willing to participate please complete the enclosed 'patient consent form' and we will arrange for you to be seen at the Royal Infirmary. Of course it is important for you to know that your participation is confidential and that you can withdraw from the study at any time, and that this will no affect your regular treatment or care.

Once again, thank you for taking the time to read this. Advances in our understanding of medical conditions are dependant on willing volunteers like yourself, and we are very grateful for your co-operation and generosity of time.

Dr Sachin Jadhav (Clinical Research Fellow)
Dr Naveed Sattar (Clinical Senior Lecturer, Pathological Biochemistry)
Professor Stuart Cobbe (Professor of Cardiology)

Contact at Glasgow Royal Infirmary University NHS Trust
Contact 0141-211-4309 or email STJadhav@aol.com for further information

Appendix 3

TITLE :

Insulin resistance as a contributor to myocardial ischaemia independent of obstructive coronary atheroma – a role for insulin sensitisation ?

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INTRODUCTION

Myocardial ischaemia is a common cause of morbidity and mortality. Essentially, it occurs when the blood supply to the myocardium is too poor its for metabolic demands to be met. The end-artery nature of the coronary circulation, with a relative lack of anastomosis between the major coronary arteries, makes the condition more prevalent in myocardium. When the myocardial metabolic demands, in particular for oxygen, are not met, anaerobic metabolism manifests, with production of lactate, leading to symptomatic angina. At the most serious end of the spectrum, myocytes that remain ischaemic beyond a critical time period die, resulting in myocardial infarction.

The importance of type 2 diabetes mellitus is well established as a cardiovascular risk factor. The cardiovascular risk of patients with insulin resistance, with or without glucose intolerance, has become apparent as described in a recent meta-analysis, looking at hyperinsulinaemia as a surrogate marker [1]. Reaven described the forerunner of what has become known as the Insulin Resistance Syndrome (also called Metabolic Syndrome X) in 1988. Since then, the definitions have been extended such that a cluster of inter-related cardiovascular risk factors including central adiposity, hypertension, dyslipidaemia and disturbances of fibrinolysis, with abnormalities of insulin metabolism at the core, are now described [2].

Here we collate available evidence to suggest that insulin resistance, in addition to promoting physical obstructive atheromatous coronary disease, may lead to myocardial ischaemia via the process of endothelial dysfunction. Based on these observations, we suggest that amelioration of insulin resistance, whether by

established or novel mechanisms, may at least in part restore normal endothelial function and potentially ameliorate anginal symptoms.

Evidence for endothelial dysfunction as a causative factor in angina independent of obstructive disease

In conventional angina there is evidence to suggest that endothelial function in the myocardial vascular bed is abnormal. Coronary atherosclerosis is associated with a reduced vasodilator response and a paradoxical vasoconstrictor response to acetylcholine. This has been shown in response to both atrial pacing and bicycle exercise during coronary angiography and these functional abnormalities may contribute to ischaemia in patients with obstructive coronary disease [3-5]. However, it is difficult to implicate this as a direct cause of ischaemia due to co-existing flow-limiting atheroma.

A subgroup of patients with angina has normal coronary arteries despite evidence of ischaemia demonstrated using non-invasive methods. This defines the group termed to have microvascular angina. There is good evidence to support the notion that these patients also have impaired microvascular function mediated by abnormal endothelial vasomotor responses and that this could potentially be an aetiological factor in ischaemia. Impaired endothelial function has been demonstrated in subjects with "Cardiac Syndrome X", using methods including brachial artery flow-mediated dilatation [6 7]and abnormal coronary vascular responses assessed by intracoronary doppler [8 9]. In fact, recent data published in abstract from support a correlation between levels of endothelial dysfunction and ST-segment depression as a marker for ischaemia in such women [10].

It is postulated that this impairment of endothelial function results in failure of the normal coronary vasodilatory response at times of stress, such that the arterial supply does not match the enhanced demand, leading to the activation of anaerobic metabolism and ischaemic pain. This group of patients provides an opportunity to study functional vessel abnormalities in the absence of obstructive atheroma.

The Link between Insulin Resistance and Endothelial Dysfunction

Insulin resistance, as well as being the precursor for type 2 diabetes, has several 'pleiotrophic' effects. These include several of the features of the metabolic syndrome including dyslipidaemia, as well as direct promotion of atheroma production.

Another such effect is its influence on vascular function. Impaired endothelial function in type 2 diabetes has been shown using venous occlusion plethysmography [11 12], high resolution brachial artery ultrasound [13], and more recently by laser doppler imaging [14]. In addition, Balletshofer et al have shown that clamp-derived indices of insulin resistance correlate with endothelial responses in first degree relatives of subjects with type 2 diabetes, using brachial artery ultrasound [15]. Similarly, Jaap et al described an association between insulin sensitivity and microvascular function in non-diabetic subjects with fasting hyperglycaemia, using laser Doppler fluximetry in response to local heating [16]. Serne et al extended these observations by demonstrating that microvascular function as assessed by laser Doppler flowimetry is associated with insulin sensitivity even in normal subjects [17]. Furthermore, in a study of the coronary circulation in subjects with unobstructed coronaries, a strong correlation between clamp-derived indices of insulin resistance and coronary vascular function assessed by Doppler blood-flow recordings was noted [18].

Other aspects of the insulin resistance syndrome have been associated with endothelial dysfunction. Obese individuals without diabetes have endothelial dysfunction both in the peripheral and coronary circulation [19] and a specific link to central adiposity has been noted by some [20]. Steinberg et al showed blunted leg blood flow in response to arterial infusions of methacholine in obese, insulin resistant subjects compared to controls. Importantly, whereas the production of euglycaemic hyperinsulinaemia augmented blood flow in lean subjects, this response was not apparent in obese [21]. Endothelial dysfunction has also been documented in in-vivo and ex-vivo experiments in subjects with hypertension and in association with dyslipidaemia, particularly in those subjects with high triglycerides and low HDL-cholesterol [22-24].

A close correlation between insulin sensitivity and endothelial nitric oxide (NO) synthesis is seen in healthy volunteers [25]. A similar correlation is seen between insulin sensitivity and vasoconstrictor responses to N-monomethyl-L-arginine (an NO inhibitor), in a mixed group of men including patients with diabetes, hypertension and healthy volunteers [26]. Furthermore, the vasodilating effect of insulin is amplified in the presence of metabolically active glucose in healthy subjects [27]. This observation, together with findings that insulin augments blood flow in lean subjects points towards a key role for insulin and glucose metabolism in maintaining vasodilator tone in vessels via NO. This is supported by the observation that exogenous insulin therapy appears to improve endothelial function in patients with type 2 diabetes [28].

The post-receptor signalling mediators involved in the production of NO in response to insulin in many ways parallel those which regulate insulin-mediated glucose transport via GLUT4 translocation (see figure 1). It appears that activation of phosphatidylinositol 3-kinase (PI3-kinase) is crucial in this signalling pathway. Cells expressing an inhibitory mutant of PI3-kinase have a much attenuated NO response to insulin. Downstream mediators of this effect may include protein kinase B (PKB) also known as Akt , [29] possibly by phosphorylating endothelial nitric oxide synthase (e-NOS) [30]. However, other pathways may also be involved and remain to be characterised. In particular, intracellular Ca^{+2} concentrations are affected by insulin, mainly by stimulation of the Na^{+}-H^{+} exchanger and Na^{+}-K^{+} ATPase and therefore insulin resistance may have a direct Ca^{+2}-mediated influence on vascular tone [31].

Microvascular angina, insulin resistance and endothelial dysfunction.

In parallel with the above observations, several groups have demonstrated relative insulin resistance in postmenopausal women and non-obese men with microvascular angina, together with lipid perturbances and higher blood pressure [32-36].

Furthermore, there are data to suggest that subjects with microvascular angina have a reduced whole-body nitric oxide response to insulin [37]. This leads to the hypothesis that resistance to this effect of insulin could manifest in impaired endothelial function via intermediates including NO.

Can improving insulin resistance improve endothelial function?

Whilst the above observations are compelling, proving a causal relationship between insulin action and endothelial function requires intervention studies. Very recent uncontrolled data show that intentional weight loss by means of a hypocaloric diet

combined with exercise, and in some cases surgical liposuction, improves endothelial vasomotor function as measured by haemodynamic responses to intravenous L-arginine (NO precursor) [38] .This improvement coincided with reduction in fasting insulin and cytokine levels.

Metformin, the only biguanide in clinical use, has been available in the UK for over 30 years. Its exact mechanism of action remains unclear but it is thought to involve increasing tyrosine kinase activity in the insulin receptor with intracellular effects mediated via the PI3- kinase pathway on GLUT4 translocation as well as NO production (figure 1).

One 12-week trial of metformin compared to placebo in individuals with type 2 diabetes showed improved endothelial function as measured by forearm plethysmography, in the metformin group alone [39]. Data recently published in abstract form, reported significant improvements in endothelial-dependant microvascular function , in a group of women with Cardiac Syndrome X, following 8 weeks of metformin treatment compared with placebo [40]. These observations concur with preliminary animal data where chronic metformin therapy improved aortic vascular properties in hyperinsulinaemic, fructose-induced hypertensive rats [41]. Metformin has other complex intra-cellular actions, one of which is activation of adenyl mono-phosphate kinase (AMP-kinase) [42], which in turn has been shown to directly activate nitric oxide synthase [43]. This mechanism in itself may contribute to the vasoactive effects of metformin independently of its action on insulin metabolism.

Modest weight loss during metformin therapy is well-described [44] and thus it is difficult to dissect whether vascular effects are solely due to this. By contrast, thiazolidinediones improve insulin sensitivity without reducing weight, with redistribution of adipose tissue from visceral to subcutaneous deposits [45]. Troglitazone has been reported to improve endothelial function measured by flow-mediated brachial artery diameter, after either four weeks [46] or four months of therapy [47]. Preliminary data have extended these findings to include an improvement in myocardial blood flow, measured non-invasively using positron-emission tomography, in non-diabetic insulin resistant individuals after three months of troglitazone therapy [48]. In animal models, rosiglitazone improved indices of insulin resistance and myography-based measures of vascular function in fatty Zucker rats [49]. There is one report, however, showing a lack of effect to troglitazone on vascular function assessed by plethysmography [50].

Can improving insulin resistance lessen anginal frequency?

Preliminary data suggests that in diabetic patients with angina , four months treatment with troglitazone not only improves endothelial function, as assessed by brachial artery ultrasound, but also treadmill exercise capacity [47]. In a study published so far only in abstract form, troglitazone has also had beneficial effects on both the frequency of chest pain, and measures of endothelial function, in vasospastic angina [51], which is associated with insulin resistance and abnormal vascular function [52]. Another study, also in abstract form so far, demonstrated improved Duke Score ischaemic measures in women with Cardiac Syndrome X following metformin administration[40]. Finally, a non-randomised study of metformin, given post myocardial infarction, to a large group of patients with varying glucose tolerance,

suggested a significant lessening of anginal symptoms and fewer new infarcts in those who received metformin compared to those who did not [53].

Conclusion

Prospective data have established markers of insulin resistance as an independent cardiovascular risk factor. We have suggested that insulin resistance is implicated in the pathogenesis of endothelial dysfunction, and therefore via this route, myocardial ischaemia. Indeed, there is tantalising evidence to show that insulin sensitisers may improve not only insulin resistance and endothelial function, but also anginal symptoms and exercise capacity in diverse patient groups. We suggest, therefore, that further properly randomised studies are required to elaborate the potential of insulin-sensitising agents in the treatment of angina.

Acknowledgements

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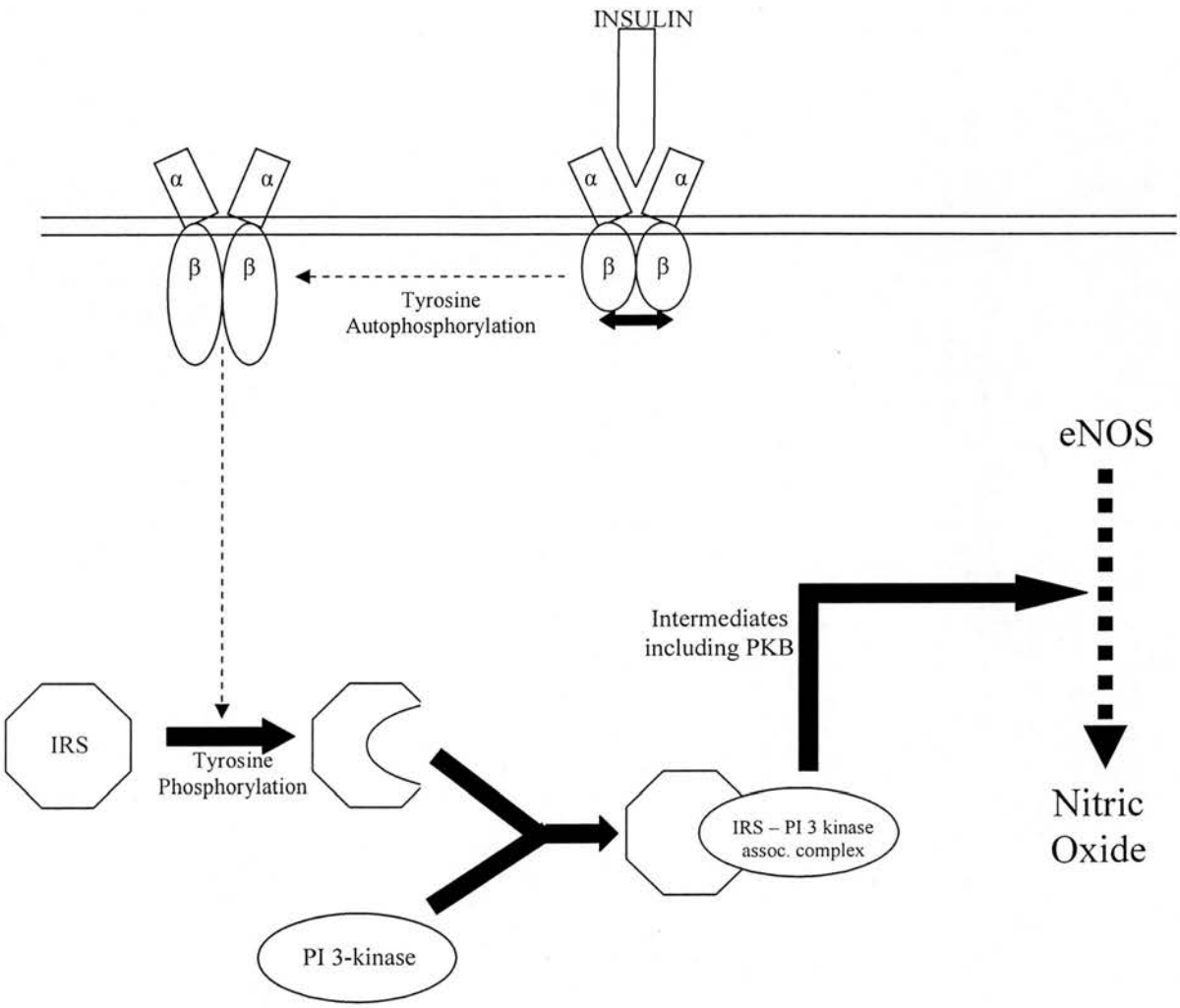
Table 1 : Summary of intervention trials looking at insulin sensitisers and outcomes of endothelial function and symptoms.

Author	Species	Population	Design	Numbers	Drug	Period	Insulin Resistance	Vascular Function	Angina
Walker et al [49]	Rat	Fatty, Zucker rats	Controlled, parallel treatment	n=8 8 controls	Rosiglitazone (50micromol/kg)	9-12 weeks	Improved (fasting hyperinsulinaemia)	Improved (ex-vivo myography)	-
Verma et al [41]	Rat	Fructose-induced hypertensive rats	2 parallel treatment groups (non controlled)	n=11 10 normal rats	Metformin 500mg/kg/day	8 weeks	Improved (fasting serum insulin/gluc)	Improved (Reactivity of aorta ex-vivo)	-
Vehkavaara et al[28]	Human	Type 2 diabetes on metformin	Controlled, parallel treatment	n=18 6 controls (remaining on metformin alone)	Insulin	6 months	-	Improved (Venous occlusion plethysmography)	-
Mather et al [39]	Human	Type 2 diabetes (diet controlled)	Placebo-controlled	n=29 15 controls	Metformin 500mg Bd	12 weeks	Improved (HOMA-IR*)	Improved (forearm plethysmography)	-
Sgambato et al [53]	Human	Post MI (mixed glucose tolerance)	Controlled (no placebo) and non randomised	n=187 123 controls	Metformin 1-2g/day	3 years	-	-	Reduced symptoms and fewer MI
Jadhav et al [40]	Human	Women with Cardiac Syndrome X	Double-blind, randomised, placebo-controlled	n=24 22 controls	Metformin 500mg Bd	8 weeks	Improved (fasting serum insulin)	Improved (laser Doppler imaging)	Improved treadmill Duke Score
Watanabe et al [46]	Human	Non-diabetic hyperinsulinaemic males	2 parallel treatment groups	n=7 8 normal subjects	Troglitazone 400mg/day	4 weeks	Improved	Improved (Brachial artery ultrasound)	-
Jack et al[50]	Human	Obese	Randomised, double blind, cross-over	n=15	Troglitazone 400mg/day	8 weeks	Improved	No change (Venous occlusion plethysmography)	-
Campaloni et al [48]	Human	Insulin resistant, non-diabetic	Non-controlled	n=9	Troglitazone 600mg/day	3 months	Improved (glucose clamping)	Improved (cold pressor test using PET [1])	-
Murakami et al [51]	Human	Diabetic with vasospastic angina	Non-controlled	n=10	Troglitazone 400mg/day	4 months	Improved (fasting hyperinsulinaemia)	Improved (Brachial artery ultrasound)	Reduced Anginal episodes and use of nitrates
Murakami et al [47]	Human	Type 2 diabetes with angina	Randomised, controlled parallel treatment	n=11 11 controls	Troglitazone 400mg/day	4 months	Improved (HOMA index)	Improved (Brachial artery ultrasound)	Improved treadmill effort tolerance

*** - HOMA (IR): homeostasis model assessment of (insulin resistance)** **1 – PET : positron emission tomograph**

Fig 1
Schematic outlining insulin receptor and
NO production coupling in endothelium



IRS : insulin receptor substrate
PI 3-kinase : phosphatidylinositol 3-kinase
PKB : protein kinase B
eNOS : endothelial nitric oxide synthase

Microvascular Function, Metabolic Syndrome and Novel Risk Factor Status in Women with Cardiac Syndrome X

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ABSTRACT

To characterise microvascular function, candidate risk pathways and metabolic syndrome prevalence in women with cardiac Syndrome X, 52 non-diabetic women with angiographically normal epicardial arteries but >1mm of planar ST depression during exercise testing (cases) and 24 healthy controls of similar age were recruited. In addition to fasting blood samples and anthropometric measures, forearm cutaneous microvascular function following iontophoresis of acetylcholine and sodium nitroprusside was assessed by laser Doppler imaging. Despite body mass index (BMI) correction and a higher proportion on statin therapy, cases had elevated insulin ($p=0.016$), triglycerides ($p=0.018$), Intercellular adhesion molecule (ICAM-1) ($p=0.021$), von-Willebrand factor (vWF) ($p=0.005$), leptin ($p=0.005$) and lower high density lipoprotein (HDL)-cholesterol ($p =0.042$) relative to controls. Consistent with these data, 30% of cases but only 8% of controls fulfilled National Cholesterol Education Program (NCEP)-defined metabolic syndrome ($p=0.015$). Finally, both endothelium-dependent and independent microvascular function were markedly impaired in the cases ($p <0.001$) and odds ratio for cardiac Syndrome X was 7.38 (95%CI 2.2 to 24.7) if acetylcholine response was < 8710 flux units. In conclusion, women with cardiac Syndrome X more commonly have metabolic syndrome and related adiposity/metabolic /inflammatory derangements. They also have significantly impaired skin microvascular function as assessed by laser Doppler imaging, consistent with generalised vascular dysfunction, a finding with potential diagnostic implications.

The objectives of this study were: i) to assess whether endothelial function using a novel non-invasive technique recently optimised in our laboratory (1;2) can discriminate between women with cardiac Syndrome X (cases) and healthy controls; ii) to determine whether circulating markers of several novel candidate risk parameters were elevated in such cases; and, iii) to determine the proportion with metabolic syndrome defined by the National Cholesterol Education Program (NCEP).

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We recruited non-diabetic women with normal epicardial arteries and an electrically positive exercise tolerance test (ETT). From 2630 diagnostic coronary angiograms performed at Glasgow Royal Infirmary between 1998-2001 we identified 226 women with normal coronary arteries. From examination of case records, 71 had a positive ETT with >1mm of flat ST depression. Exclusion criteria included type 2 diabetes (2 patients), uncontrolled hypertension (1 patient), left ventricular hypertrophy (1 patient), valvular heart disease, age greater than 70 years and significant renal or hepatic impairment. Suitable candidates were contacted by post and 52 participants agreed to enrol. Anti-anginal and anti-hypertensive medication was minimised and in most cases stopped for the duration of the study **(table 1)**. The local ethical review committee approved the protocol. Twenty-four healthy age-matched control subjects were recruited locally by advertisement. The investigation conforms to the principles outlined in the Declaration of Helsinki.

All subjects attended Glasgow Royal Infirmary fasting and had venous blood samples obtained from the antecubital fossa. These were immediately centrifuged at 2000rpm for 10 mins with the serum then being transferred into aliquots for storage at -80 °C. Analysis for lipid profile (beta-quant) and fasting glucose was undertaken

immediately. All other analyses were undertaken at the end of the study in batches. Blood pressure was measured using an Omron semi-automated in the supine position in triplicate and a mean was recorded. Waist, hip, height and weight measurements were recorded to calculate anthropometric parameters.

Microvascular function was assessed using laser Doppler imaging of the forearm cutaneous vascular bed with the subject relaxed in the supine position in a quiet, temperature and light-controlled environment, as described previously (3). Perspex iontophoresis chambers were applied to the extensor forearm surface. The cathode chamber was filled with a 1% sodium nitroprusside (SNP) solution (dissolved in 0.5% sodium chloride) and the anode chamber 1% acetylcholine (ACh) solution (also dissolved in 0.5% sodium chloride). Incremental current was applied with concurrent laser Doppler imaging to record the perfusion response. The laser Doppler imager (Moor Instruments Ltd) made use of a red laser (wavelength 633nm, power 1mW, beam diameter 1mm). The voltages across the circuit were recorded to enable calculation of the skin resistance as previously described (2). The perfusion response in flux perfusion units was calculated using Moor Instruments software package and is presented as both area under the curve (AUC) for the scan and as a plot of flux perfusion units against cumulative charge

Samples were analysed for tissue plasminogen activator (tPA), vWF (von Willebrand factor), fibrin D-dimers, intercellular adhesion molecule (ICAM-1), vascular cell adhesion molecule (VCAM), sensitive C-reactive protein (CRP), leptin and insulin. t-PA, fibrin D-dimer (Biopool, Stockholm, Sweden), and vWF (Dako, Copenhagen, Denmark) antigens were measured by ELISA techniques. The adhesion molecules were assayed using commercially-available ELISAs quantification (R&D systems Inc., Oxon UK) and C-reactive protein (CRP) (by double antibody sandwich

ELISA with rabbit anti-human CRP and peroxidase conjugated rabbit anti-human CRP: DAKO A/S, DK-2600 Glostrup, Denmark). Standard curves for CRP measurement were linear up to 5mg/l and logarithmic thereafter. The inter-assay and intra-assay coefficients of variation were less than 7% across the range of measured results. Leptin was measured by radioimmunoassay and insulin was measured by a Microparticle Enzyme Immunoassay (Abbott Laboratories) assay with a coefficient of variation (CV) <8% and sensitivity of 0.8mU/l. Plasma glucose was measured using the glucose oxidase method (Glucose Reagent Kit - Olympus AU5200, Olympus Optical Co Ltd). Finally, lipid profile was measured using established methods (Boehringer reagent kits, East Sussex, UK) in a laboratory meeting CDC standardisation criteria.

All non-parametric data were logarithmically transformed. Comparison was then made using a 2-sample t-test or ANOVA. Data are presented as mean and standard deviation (SD). Adjustment for body mass index (BMI) was made by linear regression analyses. The index of insulin resistance used was the 'Quicki' index (1/[(log insulin) + (log glucose)]). Log (fasting insulin) is also presented. The percentage of cases and controls with metabolic syndrome were compared using the Chi-square test. Receiver operating characteristic (ROC) analyses were performed to determine the areas under the curves as a measure for comparing the predictive ability for identifying potential cases of BMI and ACh response and of combination of BMI and ACh response.

The cases and controls were similar in age, menopausal status, use of hormone replacement therapy and in the proportion of smokers. Significantly ($p<0.05$) more cases than controls had had a previous hysterectomy and a history of hypertension during pregnancy (**Table 1**).

The results of classical and novel candidate risk parameters are given in **Table 2**. Log insulin was lower and the Quicki index was significantly higher in the women with cardiac Syndrome X. The cases also had higher systolic blood pressure, triglycerides and lower HDL-cholesterol relative to controls despite significantly more being on statin therapy. Moreover, serum concentrations of t-PA, vWF, V-CAM and I-CAM-1 were also higher. No difference in D-dimers was detected. The cases had significantly higher BMI, and waist circumference along with significantly higher serum leptin. Raised inflammatory indices as reflected by a higher sensitive CRP, were noted in the cases.

After adjustment for BMI (**Table 2**), between group differences persisted in insulin ($p=0.016$), systolic blood pressure ($p=0.017$), triglycerides ($p=0.018$), HDL-cholesterol ($p=0.042$), vWF ($p=0.005$), I-CAM ($p=0.021$), and leptin ($p=0.005$).

Marked differences were seen in peripheral microvascular function with endothelium-dependent (ACh) responses being attenuated in the cases as manifest by AUC, before and after correction for skin resistance ($p<0.001$) (see methods), and by 2-way ANOVA analysis of perfusion response against cumulative charge ($p<0.00001$, **Figure 1**). Similar results were seen with SNP (endothelium-independent) with a significant difference again with both AUC, raw and resistance-corrected ($p<0.001$) and 2-way ANOVA ($p<0.00001$). The differences in AUC values remained significant even after regression analysis to correct for the differences in BMI between the 2 groups. The mean AUC and 95% confidence intervals are shown in **Table 2**.

To test the potential clinical utility of microvascular function, we constructed ROC curves as a summary of the sensitivity and specificity of ACh response in differentiating cases from controls. The area under the ROC curve for ACh response

was 0.782, which compared favourably with the value for BMI (0.732). Interestingly, the combination of BMI and ACh response increased prediction to 0.824. Finally, we determined that the LDI response to ACh that best differentiated cases from controls was 3.94 log units (=8710 flux units). The likelihood odds ratio of being a case with cardiac Syndrome X below this cut-off was 7.38 (95% CI, 2.20 to 24.7).

There were more smokers in the cardiac Syndrome X group as compared with the controls, although this difference was not statistically significant (**see Table 1**). However, since smoking can attenuate vascular function, 2-way ANOVA analysis was repeated with all smokers excluded. Once again, case-control differences in both ACh and SNP-mediated microvascular responses remained highly significant (data not shown). Finally, the prevalence of metabolic syndrome as defined by NCEP was nearly four-fold higher in women with cardiac Syndrome X as compared to healthy controls ($p<0.05$, **Figure 2**).

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Our data demonstrate that despite glucose concentrations within normal ranges, women with cardiac Syndrome X have biochemical indices reflecting relative insulin resistance (4-6). We made use of fasting insulin because others have demonstrated good agreement between fasting insulin and clamp-derived indices of insulin resistance, especially in obese populations (7).

Previous work has shown that patients with cardiac Syndrome X exhibit other characteristics of the metabolic syndrome namely hypertension(8) and dyslipidaemia (9), as well as some serum markers of abnormal endothelial function (9;10). Our

results confirm the differences in blood pressure and some lipid parameters (despite more women in the cardiac Syndrome X group taking statins). However, our data are unique in a number of respects in that we are the first group to extend these findings, controlling for BMI, to abnormalities of novel independent coronary heart disease risk markers linked to insulin resistance and endothelial function, namely leptin, tPA, vWF and ICAM-1. CRP was also higher in absolute terms but BMI adjustment reduced the difference ($p=0.07$) in accordance with the known effects of adiposity on inflammatory status. Furthermore, the prevalence of NCEP-defined metabolic syndrome was more than three-fold higher in cases than controls.

We noted that 27% of the cases (vs 4% of controls, $p=0.021$) reported problems with hypertension during pregnancy. This finding suggests that impaired peripheral microvascular function is associated with hypertensive complications during pregnancy. Recent data from our group and others have confirmed that women with a history of pre-eclampsia have impaired microvascular function by the techniques of flow-mediated dilatation (11) and laser Doppler imaging (12).

Significantly, vascular impairment in women with cardiac Syndrome X extends to the microvasculature of forearm skin as evidenced by the non-invasive technique of laser Doppler imaging with iontophoresis. This is a novel technique which has been used to demonstrate microvascular impairment in a number of other related areas including diabetes (13), hypertension (14) and dyslipidaemia (15).

Our data complement the existing results in patients with cardiac Syndrome X, obtained using plethysmography and brachial artery ultrasound (16-18) but is unique in demonstrating endothelium-independent impairment compared to healthy controls. This suggests that there may be a more generalised disorder of vascular smooth muscle in these patients rather than just dysfunction at the level of the endothelium.

Previous work has relied on the administration of sublingual nitrate in drawing negative conclusions about endothelium-independent responses, whereas our data were obtained by direct iontophoretic application of nitrate solution, iontophoretically. This suggests that the sublingual delivery route may be inadequate when studying endothelium-independent responses of systemic vasculture and intravenous/intra-arterial administration may be necessary to draw conclusions about these responses when using plethysmography and ultrasound techniques.

More of the cardiac Syndrome X patients were taking aspirin during the study (73%) compared to none of the control subjects. Some groups have postulated that this may reduce the ACh perfusion response when using laser Doppler (19). However, data from our group shows that this effect is due to an increase in skin resistance reducing iontophoretic drug delivery, and in fact no attenuation in the ACh response is seen when correction for skin resistance is made (2), as was done in our present study. Therefore, aspirin is unlikely to account for the impairment in ACh response seen in the subjects with cardiac Syndrome X, and in any case, would not account for the differences observed in the SNP response.

In conclusion, we have made several novel and potentially clinically important observations in this study. Specifically, we have: extended the spectrum of metabolic and inflammatory derangements reported in women with cardiac Syndrome X; shown that such women more commonly have NCEP-defined metabolic syndrome; and, finally, demonstrated that they have markedly impaired skin microvascular function (both endothelium-dependent and independent) as assessed by laser Doppler imaging - suggesting the presence of generalised vascular dysfunction. We suggest that future prospective studies are required to address whether this simple non-invasive technique has clinical utility in diagnosing this syndrome.

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Table 1. ***Demographics of the patients with cardiac Syndrome X and healthy control group, all data number (percent) unless otherwise stated***

Variable	Syndrome X *(n=52)*	Healthy Controls *(n=24)*	P value
Age* (years)	55.6 +/- 2.07	52.0 +/- 3.04	NS
Body Mass Index* (kg/m^2)	28.58 +/- 1.24	25.1 +/- 1.58	0.001
Fasting Glucose* (mmol/L)	4.9 +/- 0.1	5.1 +/- 0.2	0.022
Smokers	8 (15%)	1 (4%)	NS
Hysterectomy	15 (29%)	1 (4%)	0.014
Peri/Postmenopausal	30 (58%)	16 (67%)	NS
Hypertension in Pregnancy	14 (27%)	1 (4%)	0.021
Statin therapy	21 (40%)	2 (8%)	0.003
Beta-blockers	1 (2%)	0	NS
Calcium Channel Blockers	4 (8%)	2 (8%)	NS
Nicorandil	2 (4%)	0	NS
Nitrates	5 (10%)	0	NS
Hormone replacement therapy	14 (27%)	6 (25%)	NS
Aspirin	38 (73%)	0	<0.001

* Mean +/- 95% confidence interval of mean.

Table 2. *Classical and novel risk factor parameters in cases and controls*
Mean and (standard deviation), unless otherwise stated

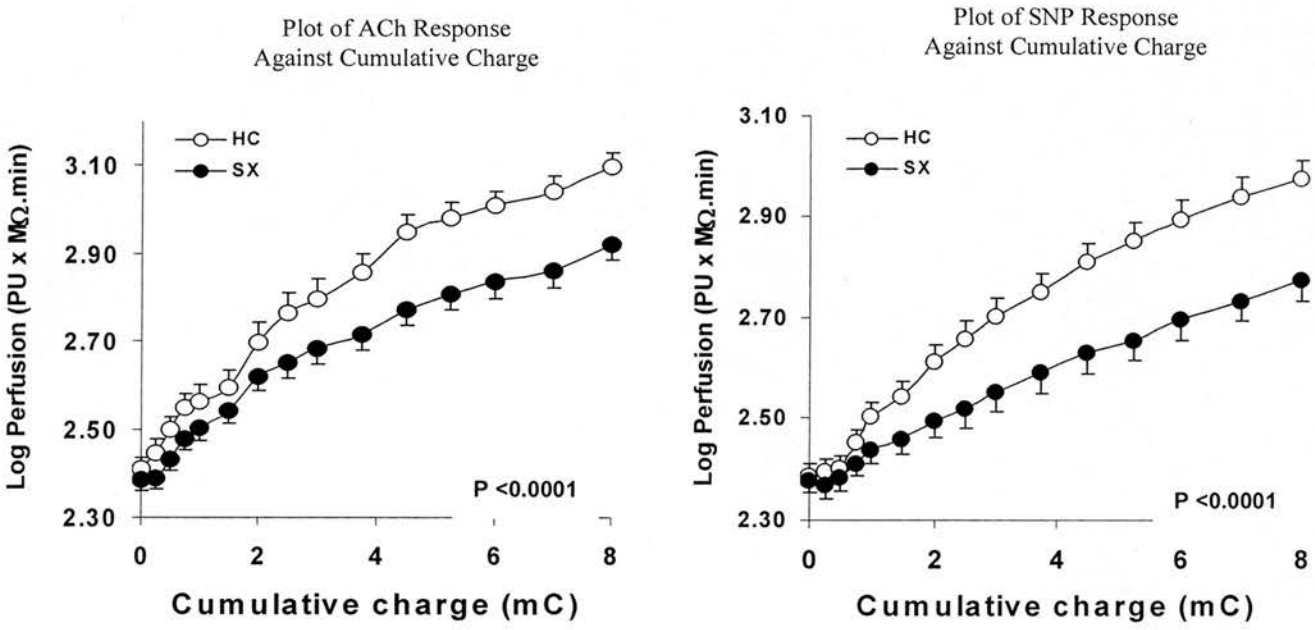
Variable	Syndrome X *(n=52)*	Controls *(n=24)*	P value	P value - BMI adj.
Total cholesterol (mmol/L)	4.97 (0.97)	4.93 (0.86)	0.839	0.838
HDL-cholesterol (mmol/L)	1.35 (0.35)	1.55 (0.35)	0.023	**0.042**
Triglyceride* (mmol/L)	1.37 (1.7)	0.93 (1.6)	0.002	**0.018**
Systolic blood pressure (mmHg)	131.6 (17.1)	119.2 (14.4)	0.002	**0.017**
Diastolic blood pressure (mmHg)	79.6 (9.0)	75.9 (7.8)	0.078	0.547
Waist (cm)	87.9 (10.9)	80.2 (8.9)	0.002	0.855
Serum leptin (ng/ml)	36.9 (14.3)	20.8 (14.3)	<0.001	**0.005**
log fasting insulin* (mu/L)	7.86 (1.70)	4.77 (1.86)	0.001	**0.016**
Quicki index †	0.65 (0.096)	0.75 (0.134)	0.001	**0.009**
Von Willebrand Factor* (%)	121.3 (1.46)	87.5 (1.56)	0.004	**0.005**
tPA (ng/ml)	7.91 (2.9)	6.13 (2.0)	0.003	0.082
ICAM-1* (ng/ml)	251.2 (1.3)	212.3 (1.2)	0.003	**0.021**
VCAM* (ng/ml)	331.3 (1.3)	290.3 (1.2)	0.012	0.067
C-reactive protein* (mg/L)	2.71 (2.9)	1.24 (2.7)	0.003	0.071
ACh (corrected) * (Units)	8375 (1.61)	13335 (1.44)	<0.0001	**<0.001**
SNP (corrected) * (Units)	7096 (1.56)	12023 (1.52)	<0.0001	**<0.001**

**Geometric mean and standard deviation. †Quicki index = (1/[(log insulin) + (log glucose)]*

HDL = high density lipoprotein, tPA = tissue plasminogen activator, I-CAM-1 = intercellular adhesion molecule

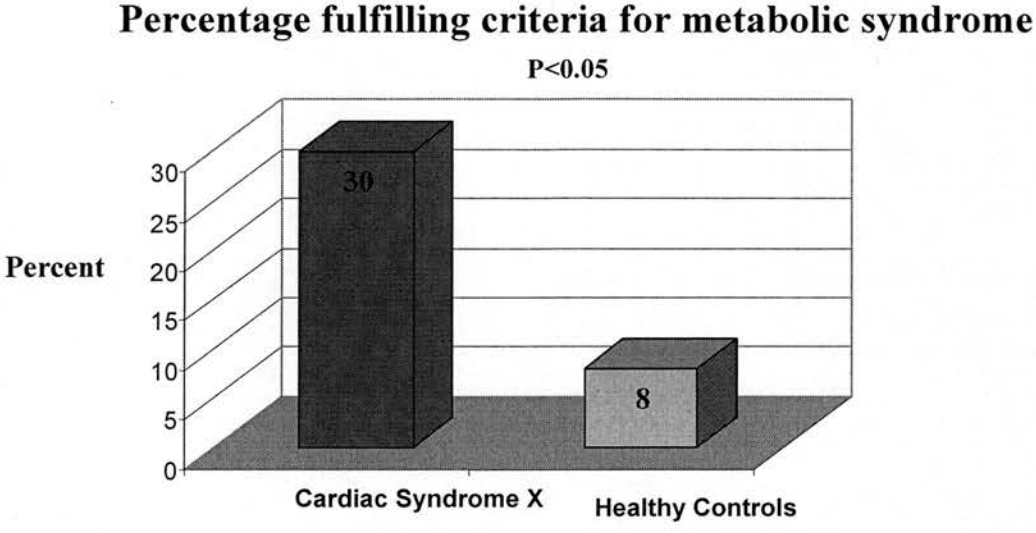
VCAM = vascular adhesion molecule, ACh = acetylcholine, SNP = sodium nitroprusside

FIGURE 1



Plots of laser Doppler imaging-assessed perfusion response to iontophoresis of acetylcholine (ACh, left) and sodium nitroprusside (SNP, right) in healthy controls (HC) compared to cardiac Syndrome X cases (SX). Values are corrected for skin resistance. The responses of both ACh and SNP are significantly different by 2-way ANOVA analysis (p values shown). Vertical bars represent the standard error of the mean.

FIGURE 2



Percent of cases and controls fulfilling criteria for metabolic syndrome based on the NCEP criteria

Effects of metformin on microvascular function and exercise tolerance in women with angina and normal coronary arteries: a randomised double-blind placebo controlled study

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Conflicts of interest: none

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ABSTRACT

Objective - to determine whether metformin, an insulin sensitizer, improves vascular function or myocardial ischemia in non-diabetic subjects

Background - Metformin prevents diabetes and may reduce coronary events in patients with diabetes but effects on microvascular function and angina are not clear

Methods We conducted an eight week double-blind randomised placebo-controlled study of metformin 500 mg BD in 33 non-diabetic women with a prior history of normal coronary angiography but two consecutive positive (ST depression $\geq$ 1mm) exercise tolerance tests (ETT). All parameters were measured at baseline and 8 weeks together with an in-vivo assessment of forearm (skin) microvascular function using laser Doppler imaging combined with iontophoresis.

Results In comparison to placebo (n=17), metformin recipients (n=16) demonstrated significant reductions in weight, and HOMA ($p<0.05$, intention to treat). Endothelium-dependent microvascular responses improved significantly with metformin (two-way repeated ANOVA, $p=0.0003$) but responses with placebo were unchanged ($p=0.50$). A comparison of change in ACh responses between metformin and placebo recipients was significant, whether analysed by a two-way ANOVA ($p<0.0001$) or change in area under curves (mean change +392 perfusion units (20 to 764)). Endothelium-independent responses were not altered. Maximal ST depression (-0.84 mm, -1.49 to -0.20, $p=0.013$), Duke score (6.1 units, 1.8 to 10.5, $P=0.008$) and chest pain incidence [-0.11 episodes per day (-0.22, 0.00); $p=0.056$] improved in the metformin relative to placebo recipients.

Conclusions Metformin may improve vascular function and decrease myocardial ischemia in non-diabetic women with chest pain and angiographically normal coronary arteries. Larger controlled trials of longer duration are warranted.

Metformin is insulin sensitizer with a history of successful use in type 2 diabetes. In the United Kingdom Prospective Diabetes Study, metformin was associated with a 39% lower risk of myocardial infarction compared to conventional therapy (1). In a more recent study, metformin was associated with a near 40% reduced total and CHD mortality when compared with sulfonylurea monotherapy (2). In a retrospective analysis of the Prevention of Restenosis with Tranilast and its Outcomes Trial (3), use of metformin in diabetic patients undergoing coronary interventions decreased adverse clinical events, especially death and myocardial infarction (79% risk reduction), when compared with patients treated with either a sulfonylurea or insulin. Consistent with such data, metformin variably improves lipids and hemostatic function in patients with type 2 diabetes (4). One placebo-controlled study in patients with type 2 diabetes suggested metformin may improve directly measured vascular function (5) independent of any weight change but such studies are lacking in non-diabetic populations.

Current therapeutic options for a well-recognised group of patients with anginal symptoms, a positive ETT but angiographically smooth coronary arteries are limited (6). The condition, sometimes referred to as "cardiac syndrome X" is not as benign as originally reported– patients presenting with unstable angina and non-obstructive atherosclerotic coronary artery disease have a 2% risk of death or myocardial infarction at 30 days follow-up (6). It is more common in women where the first presentation occurs either peri- or postmenopausally (7). Many such patients are insulin resistant (8-10) and have associated metabolic and haemostatic abnormalities (8,9). Aberrant flow mediated coronary vasomotion is pivotal in the pathogenesis of this syndrome, and occurs as part of a more generalised (systemic)

impairment in endothelial function (11). Indeed, some centres use systemic assessments of vascular function in their diagnostic pathways for this group of women (12). We recently suggested that insulin resistance, acting via endothelial dysfunction, may lead to myocardial ischemia and that insulin sensitization via metformin or other agents may lessen ischemia (13).

In the present study, we tested the hypothesis that metformin offers dual benefits of improving vascular function and lessening ischemia in non-diabetic women with angina, ischemic ETTs and normal appearing coronary arteries. Our primary end-point was the effects of metformin on microvascular function (measured by laser Doppler technology) in women previously categorised following investigation in local hospitals as having Cardiac syndrome X (i.e. angina, a positive ETT, and a normal coronary angiogram) and who also had a repeat positive ETT pre-randomisation. The secondary aims of this study were i) to assess effects of metformin on chest pain incidence, maximal ST depression and Duke score, and ii) to examine whether metformin improved other metabolic parameters linked to insulin resistance in such women.

METHODS

We recruited 33 women with smooth unobstructed epicardial arteries demonstrated on coronary angiography between 1997-2001 at Glasgow Royal Infirmary. All of these women had ongoing typical anginal-type chest pain and had previously documented $\geq$ 1mm of flat ST-segment depression on treadmill exercise testing and a repeat positive ETT at the pre-randomisation visit (i.e. all had two consecutive positive ETTs). Those with even minor coronary plaque disease, insignificant upsloping ST-segment change during exercise and coronary artery spasm at angiography were excluded. All women

underwent pre-trial screening with blood tests and echocardiography. Other exclusion criteria included age >70 years, diabetes, uncontrolled hypertension, valvular or other structural heart disease and significant hepatic or renal impairment.

Ethical approval was gained and eligible patients gave written informed consent to participate. Anti-anginal therapy was stopped or reduced for a four week period before the trial in the majority of patients, in keeping with the procedure in prior trial of hormonal replacement in cardiac syndrome X where all cardiac drugs were stopped four weeks prior to active treatment or placebo (14). After this period, a chest pain diary was provided to each woman and they were asked to record on a daily basis the numbers of typical anginal type chest pains they had suffered. We collated this information separately for four weeks prior to treatment and over the eights weeks on treatment. All patients were invited to attend in the fasting state for baseline assessments.

Laser Doppler Imaging

Microvascular function was assessed, as detailed before (15-18), using LDI with the subject relaxed in the supine position in a quiet, temperature and light-controlled environment. This was carried out before any blood tests were obtained pre-randomisation and repeated in week eight. Briefly, drugs were administered transdermally by iontophoresis, using perspex chambers applied to the extensor forearm surface. The cathodal chamber was filled with sodium nitroprusside (SNP), an endothelium-independent vasodilator (1% dissolved in 0.5% sodium chloride), and the anodal chamber with acetylcholine (ACh) an endothelium-dependent vasodilator (1% dissolved in 0.5% sodium chloride). We also employed vehicle (0.5% sodium chloride) controls in the opposite forearm. Incremental current (expressed as charge,

the integral of the current x time relationship, in milli Coulombs, mC) was applied with concurrent laser Doppler imaging to record the perfusion response. The perfusion response in flux perfusion units is presented as both the area under the curve (AUC) for the scan and as a plot of the change in perfusion with drug administration (in arbitrary Perfusion Units) against cumulative charge. The mean (± SD) between-day coefficient of variation in healthy subjects for the ACh response was 6.4% whilst the within-day, between-site coefficient of variation, measured in both forearms was 8.9%.

Blood Investigations

Fasting blood samples were collected and stored in aliquots at –80C for batch analysis at the end of the study. Tissue plasminogen activator (tPA), von Willebrand factor (vWF) (Dako, Copenhagen, Denmark) antigens, intercellular adhesion molecule-1 (ICAM-1) (R&D systems Inc., Oxon UK) and high sensitivity C-reactive protein (CRP) (DAKO A/S, DK-2600 Glostrup, Denmark) were determined by ELISA techniques. The inter-assay and intra-assay coefficients of variation were less than 7% across the range of measured results. Insulin was measured by a Microparticle Enzyme Immunoassay (Abbott Laboratories) assay with CV <8% and sensitivity of 0.8mU/l. Plasma glucose was measured using the glucose oxidase method (Glucose Reagent Kit - Olympus AU5200). Finally, lipid profile was measured using established methods (Boehringer reagent kits, East Sussex, UK). Homeostatic model assessment (HOMA, fasting plasma insulin [μU/ml] x fasting plasma glucose [mmol/l]/22.5), was used to determine insulin resistance.

Other Clinical Measures. Blood pressure was measured in triplicate following five minutes rest in the supine position using an Omron 705-CPII and the average was taken. Height and weight measurements were recorded to calculate BMI.

Exercise Tolerance Test. All patients underwent a full Bruce protocol treadmill test at baseline and then again after 8 weeks of metformin or placebo. Exercise time was noted and maximum ST-segment depression was recorded to the nearest 0.5mm to avoid artefact contamination. The Duke score [calculated as total exercise time in minutes - (5*maximal ST segment deviation) - (4*index)] was employed to provide an objective measure of treadmill-testing performance. A chest pain 'index' of 0 is assigned if no chest pain is experienced, while 1 is recorded for non-limiting pain and 2 for limiting chest pain. The Duke score has been shown to indicate prognosis in suspected coronary disease(19).

Randomisation

Metformin (500 mg tablets) and identical placebo were administered in a double-blind randomised fashion in a 1:1 fashion. Metformin and placebo were given with instructions to take one tablet daily for 1 week increasing to twice daily for the remaining 7 weeks of the trial. Randomisation was done in blocks of four with codes being held by pharmacy until data analysis was completed. Compliance was checked by pill counting. Subjects were asked to record side effects but to persevere with the tablets unless symptoms were severe.

Statistical methods

Data are presented as mean (SD) for all parameters other than chest pain incidence where median (interquartile range) are given. Comparison of change in parameter concentrations between groups was examined by the 2-sample t-test (or Mann Whitney U test for chest pain incidence) and two-way ANOVA between groups (with group and cumulative charge as main factors) for vascular function measures on an intention to treat analysis using last value carried forward in the case of drop-outs. A p value<0.05 level was considered significant. Change within groups was examined by paired t-test or Wilcoxon Sign Rank test (chest pain incidence) or two-way repeated ANOVA for vascular function (baseline versus treatment and cumulative charge as main factors). The p values reported for vascular function analyses refer to the vasodilator response to ACh or SNP at baseline compared to the value obtained following treatment with metformin or placebo (i.e. analysis confined within the treatment group), or the difference between baseline and treatment for each vasodilator when comparing metformin to placebo. Whether changes in parameters were associated was assessed using simple Pearson correlation. Adjustment for change in weight was performed by analysis of covariance.

RESULTS

Seventy-one women were screened for inclusion **[Figure 1]**. Fifty-six women with previously documented positive ETT but normal coronary angiograms were seen at a pre-randomisation visit and of these only 33 had a repeat positive ETT, and they form the study cohort. Sixteen women were randomised to receive metformin, 17 to placebo. Statistical analysis was performed as pre-specified on an "intention to treat" (ITT) basis. Compliance, as checked by pill-counting at the end of the trial, was good with 85% of study completers demonstrating >90% compliance, and compliance not

different between groups (p>0.50). Gastrointestinal side-effects were more prevalent in the metformin group affecting 10 of the 16 women compared to 3 from 17 in the placebo group ($\chi 2$, p<0.05) but were transient in the vast majority.

Demographic characteristics

The baseline characteristics are given in **Table 1**. The women randomised to metformin or placebos were similar for baseline characteristics including age, BMI and smoking history.

Primary end-point analyses on vascular function

Blood flow response to ACh improved in women who were randomised to metformin (two-way repeated measures ANOVA p=0.0003) (**Figure 2a**) but there was no significant change within placebo recipients (**Fig. 2b**). Responses to SNP were unchanged for both metformin and placebo groups (**Fig. 2c,d**). Comparing the change in ACh response (treatment minus baseline) between metformin and placebo recipients revealed a highly significant improvement in the former group relative to latter (two-way ANOVA P<0.0001), but there was no alteration for SNP (**Fig 3a,b).** Comparison of AUC in metformin relative to placebo recipients was also significant (change +392 perfusion units, 95%CI 20 to 764); p=0.04).

Metabolic effects

Analyses of metabolic changes are shown in **Table 2**. There was a significant reduction in weight and HOMA (p=0.026 and p=0.045 respectively) and trend towards reduction in t-PA antigen (p=0.09) in metformin relative to placebo recipients.

Exercise tolerance data and chest pain frequency

Baseline and follow up data for maximal ST depression and chest pain incidence in the women treated with metformin and placebo are demonstrated in **Figure 4a and 4b**. Baseline values were not significantly different between two groups for either parameter (p=0.23 and p=0.69, respectively). Significant improvements in both maximal ST depression and chest pain incidence occurred in metformin recipients (38% reduction, p=0.007 and 30% reduction, p=0.039, respectively), but such parameters were unaltered in placebo recipients (p>0.45). Maximal ST improvement was significantly larger for metformin than placebo (p=0.013); Median reduction in chest pain episodes was larger for metformin than placebo and this difference approached significance [-0.11 episodes per day (-0.22, 0.00); p=0.056]. Duke score improved by a mean of 6.1 units (95% CI 1.8 to 10.5, p=0.008) in metformin versus placebo recipients. Of the 16 women randomised to active therapy, 13 had a reduction in chest pain incidence in contrast with 6 of the 17 in placebo group (χ^2, p<0.01).

Effect of weight change on primary end-point, change in HOMA and ST depression

In the 16 women randomised to metformin alone, reduction in maximal ST depression correlated significantly only to reduction in t-PA antigen concentration (r=0.64, p=0.01) and reduction in weight (r=0.55, p=0.024), the former association independent of the latter. Changes in ACh responses and ischemic parameters did not correlate. Importantly, metformin–induced changes in ACh-mediated vascular response, HOMA and to a lesser extent ST depression were minimally attenuated by adjustment for weight change differences by group (**Table 3**).

DISCUSSION

This is the first placebo-controlled study to show that metformin improves endothelium-dependent vascular function in non-diabetic subjects. Since abnormal systemic / coronary endothelial function may be causally linked both to states of insulin resistance (20,21) and to CHD (22,23), our findings are clinically relevant and consistent with other favorable effects of metformin (1-3) and for its prevention of type 2 diabetes in subjects at elevated risk (24). Our study also suggests metformin may reduce myocardial ischemia in women with exercise-induced angina and normal coronary arteries. The combined beneficial effects of metformin on vascular function, metabolic parameters and ischemia suggest it potential to lessen vascular risk. These findings are important as the prognosis of this common condition is not as benign as previously considered (6).

We examined effects of skin microvascular function using LDI combined with iontophoresis of vasoactive agents. Our group has considerable experience with this technique, having demonstrated impaired skin microvascular function in obesity, diabetes and in the post-prandial state, whereas others have linked it to insulin resistance (15-18). The metformin-treated subjects demonstrated a clear improvement in ACh response (Figures **2a, 3a**), a finding also consistent in analyses of completers (data not shown).

Why should vascular function improve with metformin? If insulin resistance is a precursor to endothelial dysfunction, ameliorating insulin resistance should improve it. Metformin induced a small but significant reduction in weight (~1 kg or 2% relative to placebo) and a significant change in HOMA relative to placebo (>20%). Although this reduction in HOMA was slightly attenuated ($p=0.045$ to $p=0.08$) after adjusting for weight change, neither change correlated with improvement in ACh

response. However, reduction in t-PA antigen (partially endothelial-derived) concentration in the metformin group did correlate to reduction in maximal ST depression; of note, elevated t-PA is an independent predictor of both CHD events (25) and type 2 diabetes (26). Of interest, improvements in vascular function occurred despite no significant changes in glucose or inflammation parameters. De Jager and colleagues reported beneficial effects of metformin on circulating markers of endothelial function were independent of glycaemic changes in patients with type 2 diabetes (27). Changes in vascular function were also not explained by change in weight (**Table 3**). It should be noted that metformin has other complex intra-cellular actions, one of which is activation of adenosine mono-phosphate kinase (AMP-kinase) (28), which in turn has been shown directly to activate nitric oxide synthase in H-2kb cells (29) and to exert this action by phosphorylation of the endothelial isoform at serine 177 in human aortic endothelial cells (30). This is a plausible candidate mechanism for the vasoactive effects of metformin, independent of its effects on insulin action, weight or other parameters.

Our study was designed to detect change in endothelial function but we were also interested in effects on ischemia given prior - albeit uncontrolled - evidence of such benefits with metformin and troglitazone in patients with diabetes and angina (reviewed in (13)). Our positive results on effects of metformin (but not placebo) on maximal ST depression, Duke score and episodes of chest pain suggest a reduction in ischemia. As such, our findings are important and in keeping with recent novel approaches to target metabolic processes in the vasculature as a method of reducing myocardial ischemia (31). Similarly, in studies of diabetes patients, thiazolidinedione insulin sensitisers have been shown to lessen anginal pain (32), improve myocardial blood flow (33), and regulate activity of endothelial NO

synthase, the latter occuring at least in part via a peroxisome proliferator-activated receptor-gamma (PPARγ) action (34). Interestingly, a significantly lower incidence of admission for angina was reported for the pioglitazone arm relative to placebo in the recent PRO-ACTIVE study (35).

Limitations and strengths

We acknowledge that a more robust measure of myocardial ischemia would have enhanced the study – nevertheless our women had a good history of exercise angina, two consecutive positive ETTs and evidence of impaired systemic vascular dysfunction, a collection of findings, we believe, would be considered satisfactory by many cardiologists to make the diagnosis of cardiac syndrome X. Moreover, the consistency of improvements in vascular, metabolic and ischemia data by chest pain questionnaire and during the ETT in the metformin group, and in comparison to change with placebo recipients is reassuring. We also accept that although the measure of vascular function we used is well validated, it would have been optimal to measure endothelial function in coronary arteries directly. That noted, to repeat coronary angiography in a group with recently documented normal coronary arteriography would have raised ethical issues. As regards study strengths, we reiterate that, regardless of any effects on ischemic parameters, this is the first double-blind placebo-controlled study of effects of metformin on vascular function in patients without diabetes.

Conclusion

In summary, using a double-blind placebo controlled study design we have shown that metformin improves endothelium-dependent vascular function and ischemia in women with a history of exercise-induced angina, reproducible positive ETTs but normal coronary arteries on angiography. Such effects suggest that metformin may have the potential not only to improve clinical symptoms in this patient group, possibly via positive effects on microvascular function, but also to lessen their vascular risk in the longer term. Larger studies are now required to expand these novel findings.

FIGURE LEGENDS

Figure 1

The CONSORT diagram: describing outcome of all women within the study.
Footnotes:
[1] – GI side-effects
[2] – 1 patient admitted with chest pain; 1 had GI side-effects; 2 had non-specific symptoms.

Figure 2

a. Baseline and on-treatment (8 weeks) microvascular responses to ACh in the 16 women randomised to metformin (p=0.0003, two-way repeated measures ANOVA,)
b. Baseline and on-treatment (8 weeks) microvascular responses to ACh in the 17 women randomised to placebo (p=0.38, two-way repeated measures ANOVA)
c. Baseline and on-treatment (8 weeks) microvascular responses to SNP in the 16 women randomised to metformin (P=0.46, two-way repeated measures ANOVA,)
d. Baseline and on-treatment (8 weeks) microvascular responses to SNP in the 17 women randomised to placebo (P=0.95, two-way repeated measures ANOVA).

Figure 3

a. Change over 8 weeks in microvascular responses to ACh in metformin and placebo groups (P<0.00001, two-way repeated measures ANOVA).
b. Change over 8 weeks in microvascular responses to SNP in metformin and placebo groups (P=0.55, two-way repeated measures ANOVA).

Figure 4

a. Mean maximal ST depression during ETT (mm) at baseline and on-treatment (8 weeks) in the 33 women randomised to placebo (n=17) or metformin (n=16).
b. Median chest pain incidence prior to randomisation and on treatment in the 33 women randomised to placebo (n=17) or metformin (n=16).

Table 1. Baseline characteristics of 33 women with repeat positive ETT at baseline

	Metformin n=16	***Placebo**** n=17
Age (years)	***55.8 (8.8)***	***58.1 (8.4)***
Body mass index (kg/m^2)	28.2 (3.7)	28.1 (3.6)
Weight (kg)	***71.3 (8.7)***	***72.7 (9.5)***
Current smoker, n (%)	***3 (19)***	***3 (18)***
Postmenopausal, n (%)	***10 (63)***	***8 (47)***
HRT use, n (%)	***6 (38)***	***3 (18)***
Aspirin, n (%)	***13 (81)***	***14 (82)***
Statin use, n (%)	***8 (50)***	***5 (29)***
Beta blockers, n (%)	***0 (0)***	***1 (6)***
Nitrate, n (%)	***2***	***2***
Calcium channel blockers, n (%)	***1***	***0***
Nicorandil, n (%)	***0***	***2***

*No significant difference (P>0.10) in any baseline characteristics in metformin vs. placebo group

Table 2. ***Clinical characteristics of the metformin and placebo groups at baseline and comparison of change over 8 weeks in metabolic parameters. Data are means (SDs).***

	Metformin n=16		Placebo n=17			
	Baseline	Change	Baseline	Change	Mean diff, 95% CI	P value
Weight (kg)	71.3 (8.7)	-0.64 (1.45)	72.7 (9.5)	0.48 (1.29)	-1.13 (-2.10, -0.15)	0.026
Fasting Glucose (mmol/L)	4.89 (0.5)	-0.07 (0.47)	4.87 (0.4)	0.11 (0.28)	-0.18 (-0.46, 0.10)	0.20
HOMA	1.80 (1.02)	-0.51 (0.79)	1.92 (1.32)	0.01 (0.62)	-0.52 (0.014, 1.025)	0.045
Systolic blood pressure (mmHg)	130.0 (21.0)	-5.7 (26.0)	136.5 (11.2)	0.12 (8.7)	5.8 (-8.2, 19.8)	0.40
Diastolic blood pressure (mmHg)	78.3 (8.7)	-2.4 (8.9)	81.3 (26.0)	-3.6 (6.6)	**1.2 (-4.4, 6.8)**	**0.66**
Total Cholesterol (mmol/L)	4.74 (0.7)	-0.04 (0.71)	5.27 (0.9)	-0.21 (0.68)	**0.17 (-0.33, 0.67)**	**0.49**
Triglycerides (mmol/L)	1.31 (0.5)	-0.08 (0.39)	1.40 (0.7)	0.05 (0.36)	-0.13 (-0.40, 0.14)	0.33
LDL-cholesterol (mmol/L)	2.85 (0.5)	0.06 (0.53)	3.26(0.8)	-0.13 (0.50)	0.07 (-0.29, 0.43)	0.70
HDL-cholesterol (mmol/L)	1.49 (0.3)	0.041 (0.15)	1.30 (0.4)	-0.015 (0.11)	0.06 (-0.04, 0.15)	0.23
t-PA (ng/ml)	7.00 (3.2)	-1.28 (1.93)	8.53 (3.2)	0.06 (2.5)	-1.3 (-2.9, 0.23)	0.09
Von Willebrand Factor (iu/dl)	122 (41)	7 (14)	134 (36)	8 (16)	-1.0 (-12, 10)	0.86
ICAM-1 (ng/ml)	273 (75)	-7 (40)	264 (68)	-5 (12)	-2.3 (-24, 20)	0.83
C-reactive protein (mg/l)	3.45 (2.0)	-0.10 (1.8)	3.3 (2.9)	0.31 (0.67)	-0.4 (-1.5, 0.6)	0.42

Table 3. Raw and weight change-adjusted differences in metformin versus placebo recipients for change in vascular response (AUC for ACh-mediated perfusion), HOMA and ST depression.

	Beta	SE	P
AUC for ACh response	392	179	0.04
Adjusted for delta weight	395	197	0.05
HOMA (raw difference)	-0.52	0.25	0.045
Adjusted for delta weight	-0.49	0.27	0.08
ST depression (raw difference)	-0.84	0.30	0.013
Adjusted for delta weight	-0.51	0.29	0.095

Figure 1

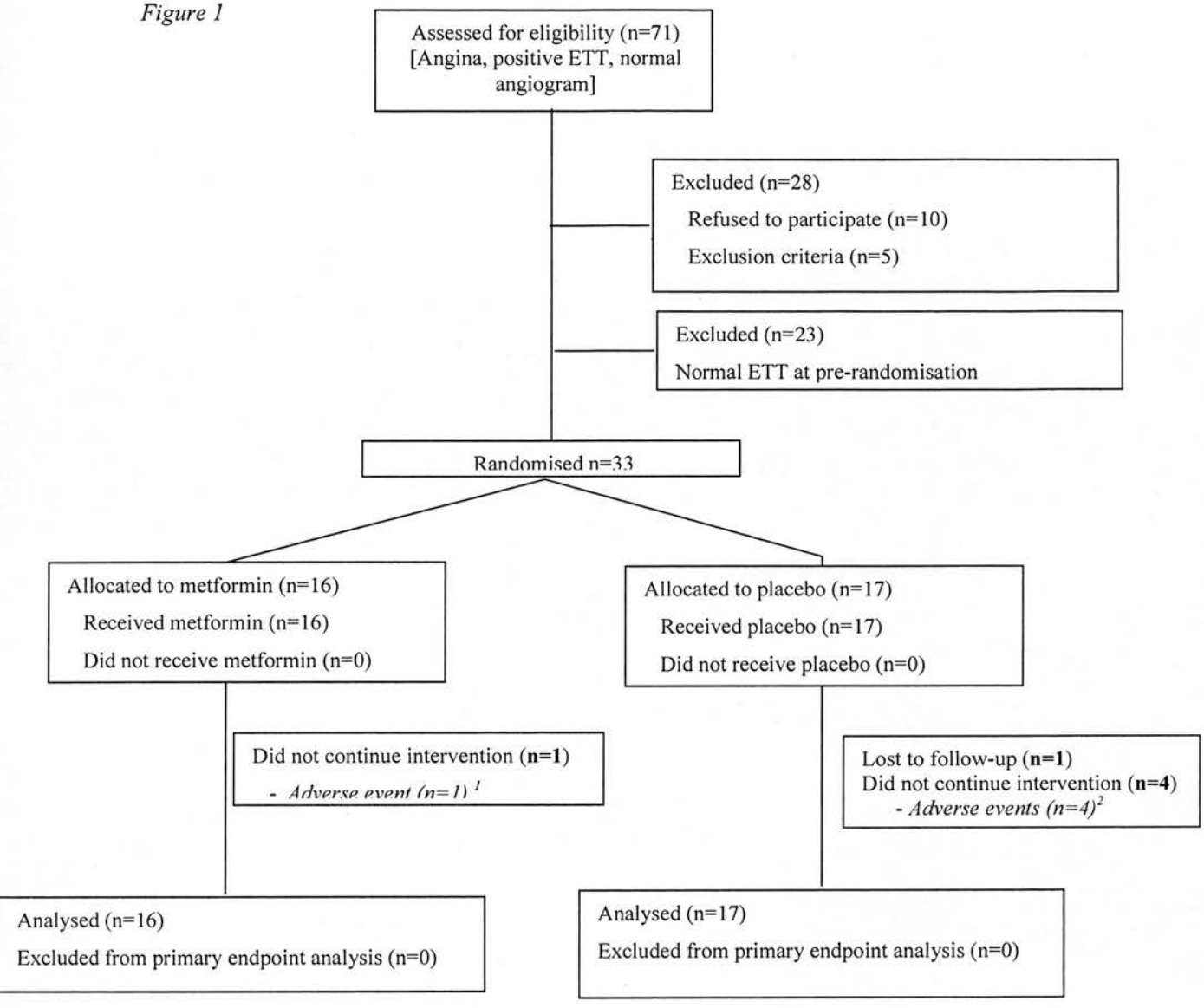

Assessed for eligibility (n=71)
[Angina, positive ETT, normal angiogram]
Excluded (n=28)
Refused to participate (n=10)
Exclusion criteria (n=5)
Excluded (n=23)
Normal ETT at pre-randomisation
Randomised n=33
Allocated to metformin (n=16)
Received metformin (n=16)
Did not receive metformin (n=0)
Allocated to placebo (n=17)
Received placebo (n=17)
Did not receive placebo (n=0)
Did not continue intervention (n=1)
- Adverse event (n=1) [1]
Lost to follow-up (n=1)
Did not continue intervention (n=4)
- Adverse events (n=4)[2]
Analysed (n=16)
Excluded from primary endpoint analysis (n=0)
Analysed (n=17)
Excluded from primary endpoint analysis (n=0)

Figure 2a,c (Metformin) & 2b,d (Placebo)

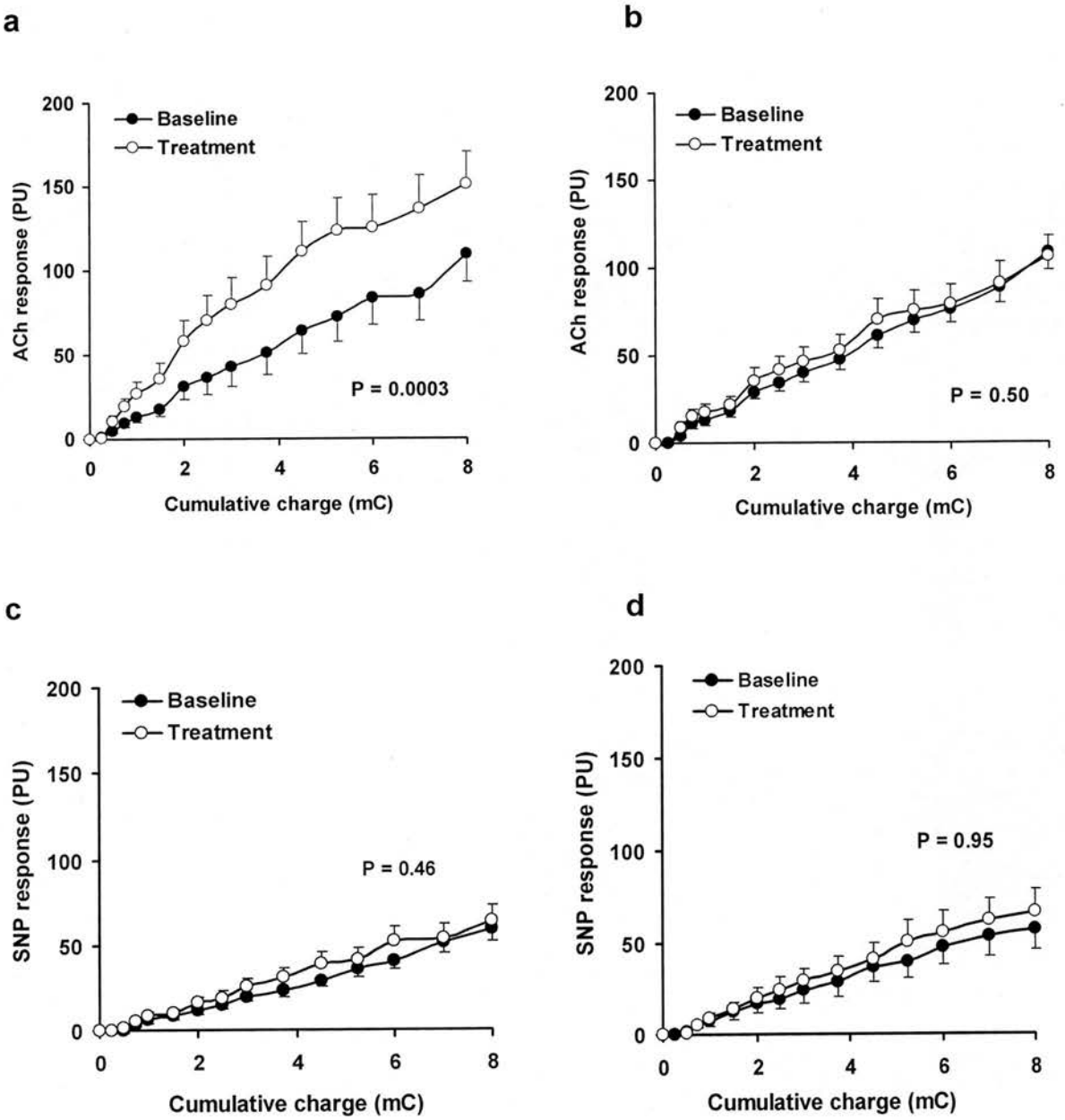
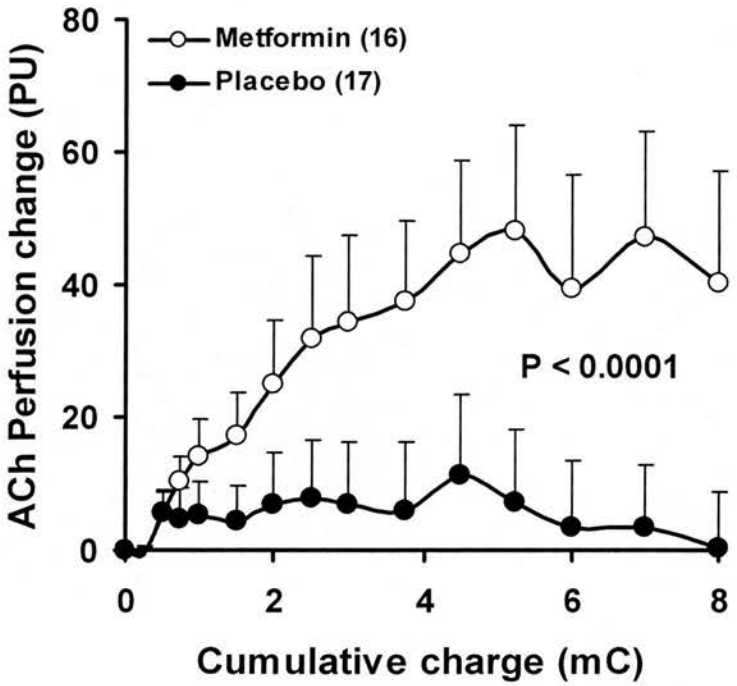


Figure 3a (ACh) & b (SNP)

a



b

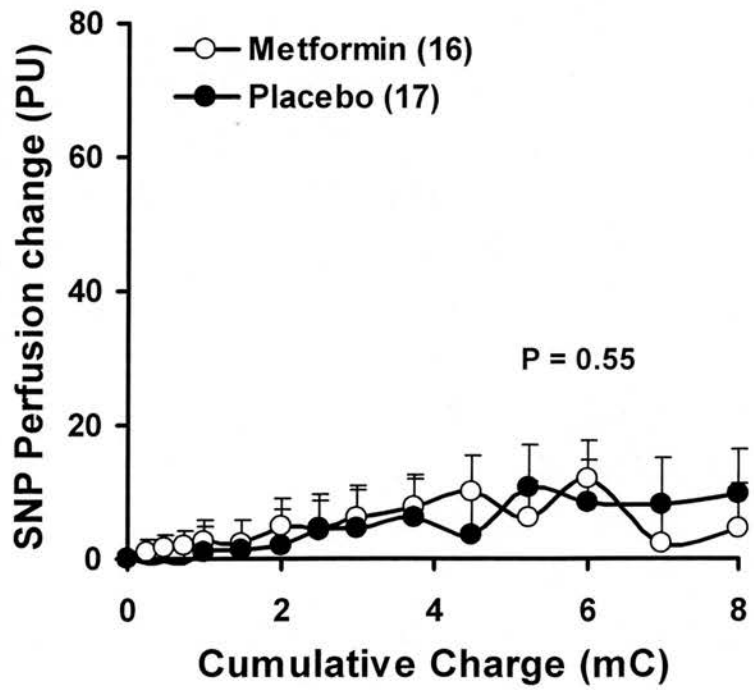


Figure 4a

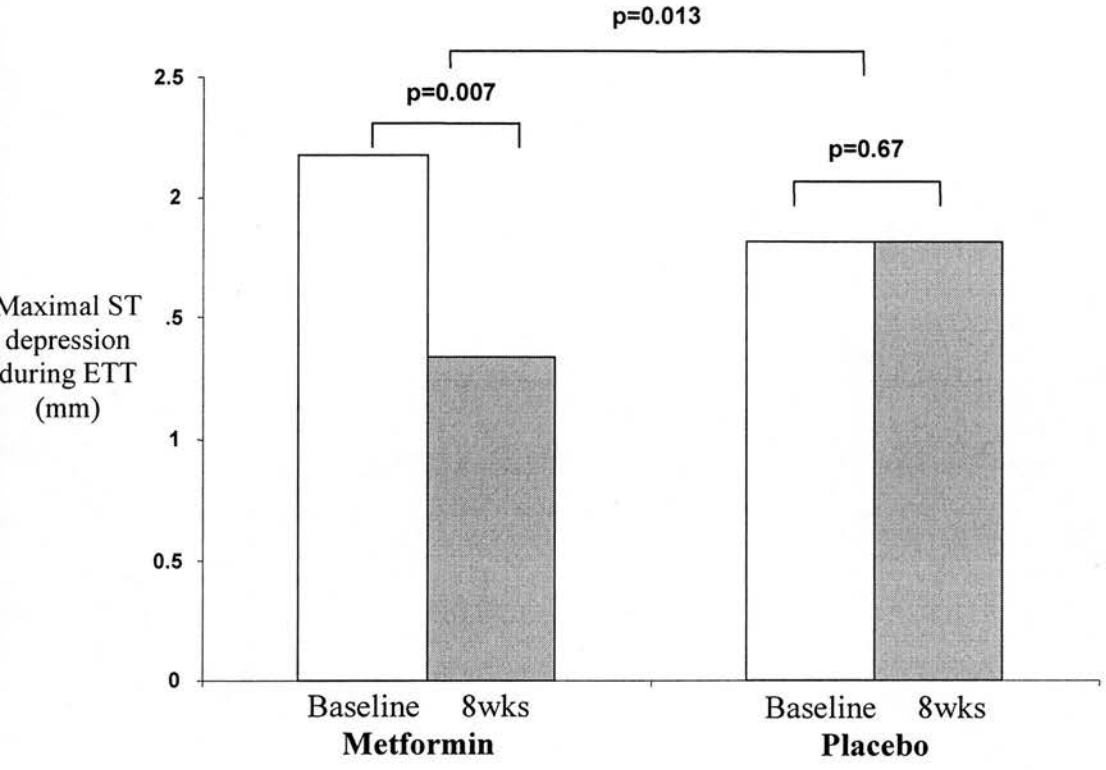
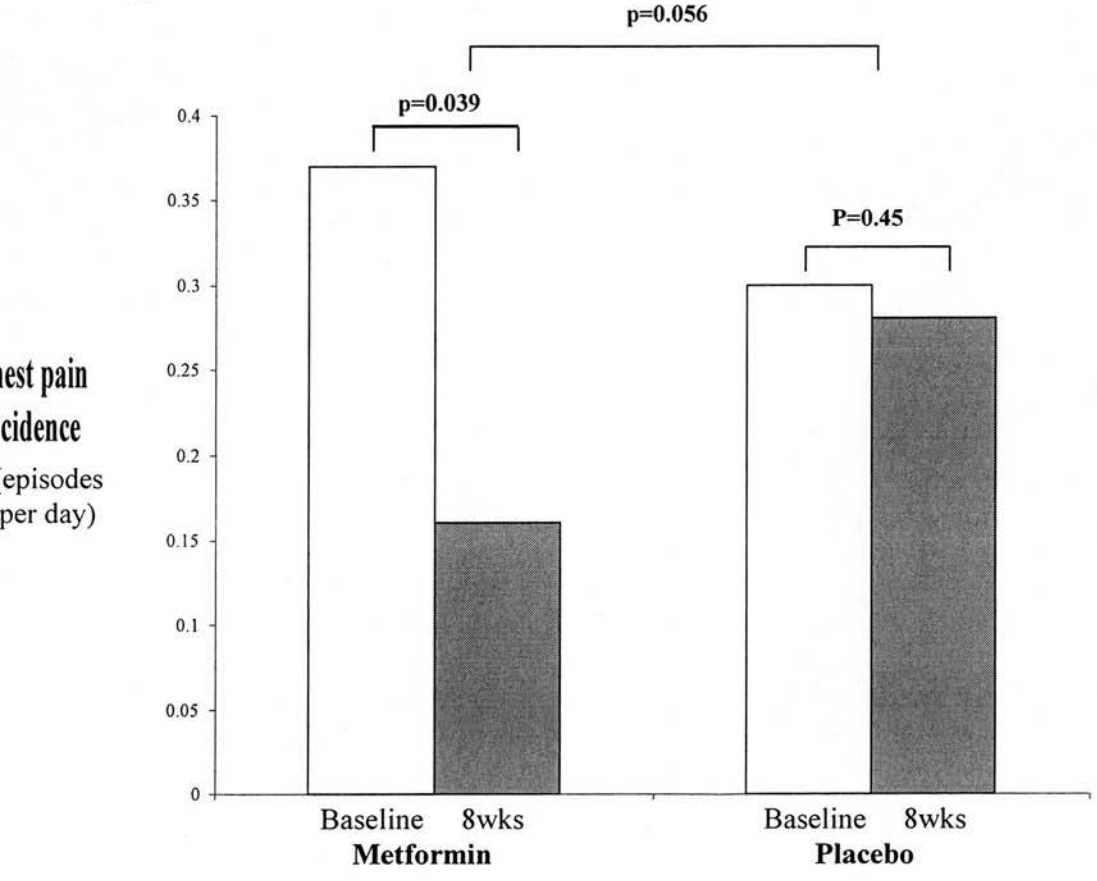


Figure 4b



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Endothelial function is negatively correlated to ST-segment depression during exercise in women with anginal chest pain and normal coronary arteries

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Background. Women with chest pain and normal coronary arteries present a common and often difficult clinical scenario. A proportion have myocardial ischaemia, caused by microvascular disease and it has been suggested that endothelial dysfunction with reduced vasodilator capacity might be an aetiological factor. We set out to look at levels of endothelial dysfunction in this group using a non-invasive technique, and compare with an age –matched control group.

Methods. We recruited 51 women with both an electrically positive exercise tolerance test (ETT) and a coronary angiogram in the previous two years demonstrating normal epicardial vessels. The ETT was repeated with manual measurement of maximal ST segment deviation. Endothelial function (EF) was assessed non-invasively in the forearm cutaneous vascular bed by means of laser-doppler imaging in response to iontophoretic application of acetylcholine (ACh) and sodium nitroprusside. An arbitrary cut-off point of 10000 units (area under the curve) for the ACh response was used to categorise subjects those with 'normal' or 'impaired' EF. Endothelial function was assessed in 25 control subjects using the same technique.

Results. 33 women in the chest pain group (65%) had impaired EF. There was a negative linear correlation between the extent of ST depression and EF ($r=0.44$ $p=0.001$). All women with greater than 2mm ST depression had impaired EF and no women with 'normal' EF had 2mm or more of ST depression. There were significant differences between the EF of women in the control group compared to women in the chest pain group ($p<0.0001$).

Discussion. There was an association between endothelial dysfunction and ST changes on ETT in women with chest pain and normal coronary arteries. EF in these women is significantly impaired compared to matched controls. Our data suggest a potential novel application of non-invasive assessment of endothelial function as an adjunct to ETT in identifying with greater sensitivity and specificity, women with and without microvascular angina.

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Women with Cardiac Syndrome X Exhibit Features of the Insulin Resistance Syndrome and Impaired Peripheral Microvascular Function.

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Background. Anginal chest pain despite angiographically normal coronary arteries in the context of a positive exercise test is a common problem. A proportion of this group, labelled as cardiac 'syndrome x' (CSX), have evidence of myocardial ischaemia. We sought to characterise women with CSX in terms of their metabolic parameters and peripheral microvascular function (MF) as compared with controls

Methods. We recruited 56 women fulfilling the criteria for CSX along with 25 healthy matched controls (HC group). Fasting blood samples and clinical measurements were obtained. Forearm cutaneous microvascular function was assessed by iontophoresis of topically applied acetyl choline and sodium nitroprusside and laser Doppler imaging

Results. There were significant differences between the CSX and HC with regards to indices of insulin resistance ($p<0.001$), systolic blood pressure ($p<0.012$), triglycerides ($p<0.01$), HDL-cholesterol ($p=0.012$), C-reactive protein ($p<0.005$), body mass index (BMI) ($p<0.001$) and serum leptin ($p<0.001$). Peripheral MF, both endothelium-dependent and independent, was impaired in the CSX group compared to the HC group ($p<0.001$) and circulating vascular markers (von willebrand factor, tissue plasminogen activator, and cellular adhesion molecules) were also different between the 2 groups ($p<0.001$). Adjusting for BMI attenuated the differences in the metabolic parameters as did adjustment for insulin. However the differences were further attenuated after correction for serum leptin. The differences in MF remained highly significant even after adjustment

Discussion. Subjects with CSX exhibited features of the insulin resistance syndrome. Attenuation of these differences after correction for leptin highlights the importance of adiposity and insulin resistance in CSX. Impairment of both endothelial-dependent and independent MF suggests not only endothelial dysfunction but more generalised vascular smooth muscle dysfunction which may be important in the aetiology of myocardial ischaemia. Adjusting for leptin and insulin did little to attenuate these differences in MF suggesting other factors play a critical role

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Effects of Metformin on Microvascular Dysfunction, Metabolic Parameters and Ischaemic Measures in Women with Cardiac Syndrome X : a Randomised Double-Blind Placebo-Controlled Trial.

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Background. Insulin resistance and associated vascular dysfunction are potential aetiological factors for Cardiac Syndrome X (CSX) - angina with positive exercise tolerance test but angiographically unobstructed epicardial arteries. We sought to determine whether metformin would reverse the metabolic derangements and vascular dysfunction, in such women, and as a result lead to less ischaemia.

Methods and Results. 56 non-diabetic women with angina, >1mm ST-segment depression on exercise testing and angiographically normal coronary arteries were recruited. Metformin 500mg twice daily was administered for 8 weeks in a double-blinded randomised placebo-controlled manner (DBRPCT). Baseline and post-treatment data were collected – fasting blood tests, full Bruce-protocol ETT, anthropometric measures. Laser Doppler imaging combined with iontophoretic application of sodium nitroprusside (SNP) and acetyl-choline (ACh) to the forearm extensor surface, was used to assess endothelial independent and dependant microvascular function.

At baseline women who took metformin (n=24) were well matched with those given placebo (n=22) in terms of age, metabolic parameters, treadmill performance and microvascular function. Metformin treatment resulted in significant reductions in fasting insulin (p=0.036), tissue plasminogen activator (p=0.026), body mass index (p=0.003), and ST-segment depression during exercise (p=0.003), and significant elevations in treadmill Duke score (p=0.001) and HDL-cholesterol (p=0.049), relative to placebo effects. Endothelial-dependant microvascular function was significantly improved with metformin group (p<0.001) with no change in the placebo group (Figure 1) but endothelial independent function did not alter.

Discussion. These data show for the first time in the context of a DBRPCT that metformin treatment can improve metabolic parameters, microvascular function and clinical outcome measures relating to ischaemic burden in women with CSX. Future studies should examine whether metformin has a potential clinical role in patients with the metabolic syndrome, who have angina and obstructive coronary disease, as vascular dysfunction is a feature in this population also.

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Figure 1.